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EDITOR'S VIEWPOINT

Takuma (p. 152) reports the results of electron microscope studies of enamel surfaces from normal and abnormal teeth. While carious surfaces show early changes, the appearance is not consistent, suggesting, again, that dental caries may represent more than one process. The author has observed caries beginning in the rods but has never seen it initiated in the interrod substance. Matsumyi and Takuma have recently published an *Atlas of Electron Micrographs of the Human Dental Tissues* (Tokyo Dental College Press, 1954). It is beautifully printed and most complete. The 139 reproductions (4½ by 6 inches) make it unequaled.

Linghorne and O'Connell demonstrated that the connective tissue could reattach to the cementum in experimentally produced periodontal pockets. Extending their studies (p. 164) they produced pockets about the teeth of dogs and simulated the disease condition in man by permitting them to become infected and epithelialized. The root surfaces then were scaled and the pockets packed. Reattachment of connective tissue to new cementum was observed even after exposure of the root surface to oral fluids and whether new alveolar bone formed or not. The authors show that the epithelial attachment may develop crownwise as well as rootwise, depending on conditions. Such studies may eventually lead to more rational periodontal therapy.

Hill (p. 178) presents the data on salivary lactobacillus counts and dental caries prevalence rates after addition of fluorides to the water of Evanston. There were definite shifts in the distribution of lactobacillus counts. The author assumes that the lactobacillus count is an indicator of lactobacillus activity, a generally acceptable concept, but the possibility remains that fluorides may interfere with lactobacillus metabolism in such a way as to reduce caries activity or that of the organisms without consistently reducing numbers.

Grace's data from the examination of 1,874,067 Pennsylvania school children have been analyzed by Kimmelman (p. 188). He has emphasized that dental restorations lag behind caries in children so that the number of unfilled decayed teeth increases annually. He points out the need for more (and better) restorative dentistry in children and for application of control measures.

Jørgensen (p. 195) has analyzed the Dryopithecus pattern in the molars of modern Danes and Dutchmen. His findings suggest racial patterns but, unfortunately, as the author points out, they do not lend themselves entirely to statistical analysis. A review of this JOURNAL in its earliest volumes indicates a greater interest in comparative dental anatomy then than is now evident. Occasional studies in this field still appear, but the vast majority of investigations are in other fields, using newer techniques. Comparative studies can still contribute much.

Brudevold and Steadman (p. 209) found 30 ppm of copper in pooled surface enamel from 30 teeth. The authors consider that copper etched out of surface enamel at pH 4 may lead to interference with the glycolytic cycle by copper ions. Copper is not readily adsorbed after eruption.

The calcification of enamel matrix has been investigated by histologic studies, embryologic experiments, microincineration, chemical analyses, Grenz rays, polarized light, and radioactive isotopes. Myers (p. 217) extended investigations into this still controversial field by using radioactive calcium salts in young rats and localizing them in the teeth and jaws with radioautographs. Comparisons of radioautographs and radiographs of the same jaws are used to localize regions of mineral exchange.

Myers (p. 225) found that at the end of 2 weeks the femurs of phosphorus-deficient animals have a ten times greater P^{32} turnover than controls, most of the difference occurring in the second week, while the incisors and molars have only a slightly

higher P^{32} turnover rate than those of controls. The findings appear to support the concept that rachitic osteoid is removed before new matrix is deposited and calcified, whereas in teeth the poorly formed dentin remains and new, normal dentin is laid down against it. In this way the tooth pattern remains as a record of the metabolic disturbance but the bone defect is largely erased.

Myers (p. 230) found that rats recovering from phosphorus deficiency show slower gain in mineral ash content of teeth than in body weight but faster increase in femoral mineral ash content than in body weight. The smaller volume of tooth structure capable of response (uncalcified dentin and osteoid) is suggested as explaining, in part, the discrepancy between histologic and chemical findings. Bone, as a vascular and cellular tissue, is very plastic and reactive, while the dentin has only limited access to vital processes.

Schwartz and Shaw (p. 239) removed various salivary gland combinations from albino rats to observe the effect in cavity formation. In these animals the parotids appeared more important but the submaxillaries were almost equal in effect. A unilateral caries increase was produced by removing glands on one side, an effect observed in human beings where the salivary flow is inhibited on one side. The protective action of saliva is well recognized but its modus operandi is still unsolved.

Woldring (p. 248) presents a chromatographic analysis of amino acids in human saliva. Microbiologic methods have been used to demonstrate 16 salivary amino acids. Woldring identified 18 salivary amino acids but his values are lower than those found by microbiologic methods, generally. Just as one uses different methods and different media for demonstration of particular bacterial species, the biochemist may combine the different assay methods for more complete information.

The collection of saliva from separate glandular groups presents a problem to the investigator. Gore (J. D. Res. **17**: 69, 1938) and others described apparatus for separation of parotid saliva, but separation of sublingual and submaxillary saliva is more difficult. Schneyer (p. 257) describes an apparatus that can be constructed in any prosthetic laboratory to permit separation of sublingual and submaxillary salivas.

James (p. 262) kept records for 2,708 local anesthetics administered in private practice. Correlating failures and toxic reactions with weather conditions showed both associated with weather changes, failures being at temperature peaks and toxic reactions at dips. A second study (p. 268) of 1,103 lidocaine anesthetics showed the same trend. With procaine, there were 5.1 per cent failures and 1.2 per cent gave toxic reactions; while lidocaine 3.7 per cent were failures and there were only 2 toxic reactions (0.18 per cent). It would be interesting to compare these data with similar information from a completely air-conditioned building and from an air-conditioned office.

Mitchell, Schoonover, Dickson, and Vacher (p. 273) used the x-ray diffraction technic on dental amalgam to observe the presence of uncombined mercury. The temperature of the amalgam was lowered to produce crystalline reaction to the x-ray. The authors propose that initial shrinkage of amalgam is due to reduced value of mercury-alloy compounds over mercury and alloy, that the subsequent expansion is due to diffusion of uncombined mercury into new areas where it can combine with alloy to create a second contraction. The authors also explain the results of overtitration on this mechanism.

Loewenstein and Rathkamp (p. 287), doubting that the nerve tissue of the tooth should be assigned the sole function of pain reception, studied its pressoreceptive ability. They recorded pressure thresholds and found significant differences between pulpless and pulp intact teeth and between teeth covered with metal caps and uncovered teeth. The thresholds are low enough to make the pressoreception highly important in controlling masticatory force and function. The indirect evidence suggests that intradental pressor receptors exist and that vertically applied pressure could aid in diagnosis of the condition of the pulp.

Turkheim (p. 295) tested the antibacterial ability of zinc oxide eugenol cements

on bacterially contaminated dentin in vitro and in vivo. His in vivo results showed bacteriostatic and bactericidal effects but, in vivo, the organisms survived. Perhaps further controlled in vivo studies are indicated, but the author proposes incorporation of antibacterial agents into the base material.

A recent publication of interest is *Viral and Rickettsial Diseases of the Skin, Eye and Mucous Membranes of Man* by Blank and Rake (Little, Brown and Company, 1954). The general considerations and specific oral references make it of value to investigators and clinicians in the dental field.

H. B. G. R.

THE ELECTRON MICROSCOPY OF THE ENAMEL SURFACES OF TEETH UNDER VARIOUS ABNORMAL CONDITIONS

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IN A series of reports[1-6] my colleagues and I have given the results of our studies of the teeth with the electron microscope. Description of the microstructure of the tooth surface has been handicapped by difficulties in observing it. Although reports have been made on observations through reflecting microscopes,[14-16] the tooth surface is too complicated and uneven for accurate studies by such methods. The shadowed replica method has made it possible to observe the surface in three dimensions by either optical or electron microscopes.[3, 4, 6-13]

This report gives the results of surface studies on abnormal teeth and some views on carious enamel.

METHOD

The shadowed methylmethacrylate or collodion-aluminum replica method was used. On the surface of the primary replica of methylmethacrylate or collodion, previous to dissolving it, aluminum is vacuum evaporated. The thin undissolved aluminum membrane is used as the specimen and is shadowed with gold or chromium.[3]

OBSERVATIONS

Abnormal Enamel Surfaces.—As previously reported,[3] the outer surface of the enamel at the rod end and at the interrod part is composed of minute particles about 0.1 to 0.5 μ in diameter. These are regarded as the units of calcification. It may be assumed that pathologic changes may influence the form of these units.

Mottled Enamel.—In the optical microscope, severely mottled enamel may show distinct rod ends and distorted perikymata inside the defect (Fig. 1), a change which can hardly be distinguished from caries.[3] In other instances the enamel surface is rough but not characteristic. Under the electron microscope large and rough particles of varying size coexist (Fig. 2) or, in very severe mottling, there may be a distinct transformation of the rod end with rough calcifications (Fig. 3). This is seen in teeth from individuals from regions with 5.6 ppm of fluorine in the water supply. In such severely mottled enamel, crystallike fragments sometimes adhere to the replica, owing to their fragility, and may be observed as a pseudo replica.

Fig. 4 shows the pronounced roughness of unerupted enamel surface found in a dog which was injected intravenously with solution containing 20 mg. of NaF per 1 kilogram of the dog's weight every day for 268 days

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starting at the age of 70 days. The erupted part of the enamel surface showed severe discoloration and defection by mastication, but the unerupted and protected part showed only chalkiness.

Fig. 1.

Fig. 2.

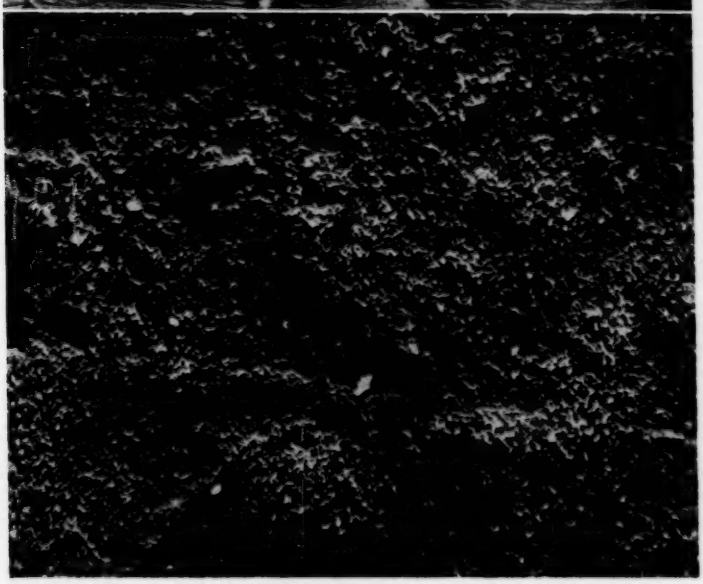

Fig. 1.—Defect noted on mottled enamel (optical micrograph) ×90. (Labial surface of upper left second incisor of 16-year-old female living in an area where the drinking water contains 5.6 ppm fluorine.)

Fig. 2.—Surface of mottled enamel ×5,800. (Labial surface of upper left incisor of an inhabitant of an area where the drinking water contains 0.6 ppm fluorine.)

The imperfect calcification of mottled enamel may be observed on its cut and polished surface, as well as on its outer surface. Fig. 5 shows the chromium oxide polished surface of mottled enamel with its clear prismatic

Fig. 3.

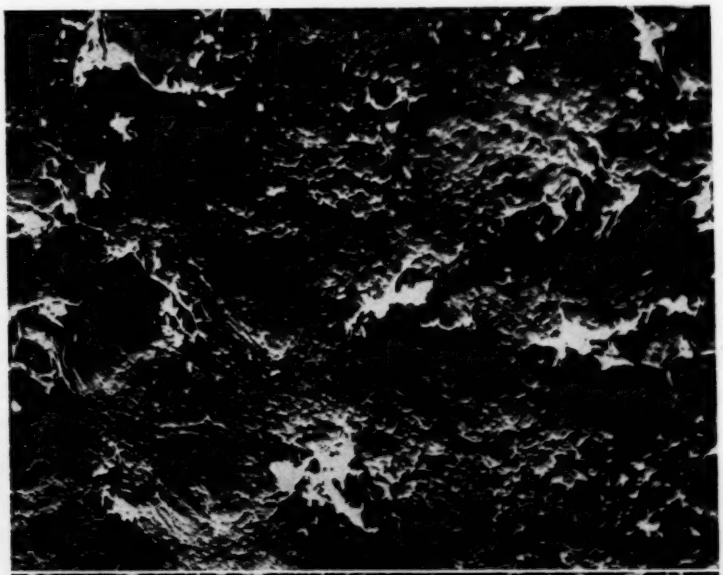

Fig. 4.

Fig. 3.—Surface of mottled enamel ×4,200. (Taken from the inside of the defect noted in Fig. 1.)

Fig. 4.—Enamel surface of experimentally fluorosed dog which was injected with NaF (×3,700).

structure and lines of Retzius in a region which would appear chalky to the naked eye. When such structural detail is observed on the cut and polished surface of normal enamel, it is usually near the dentinoenamel junction, not

Fig. 5.

Fig. 6.

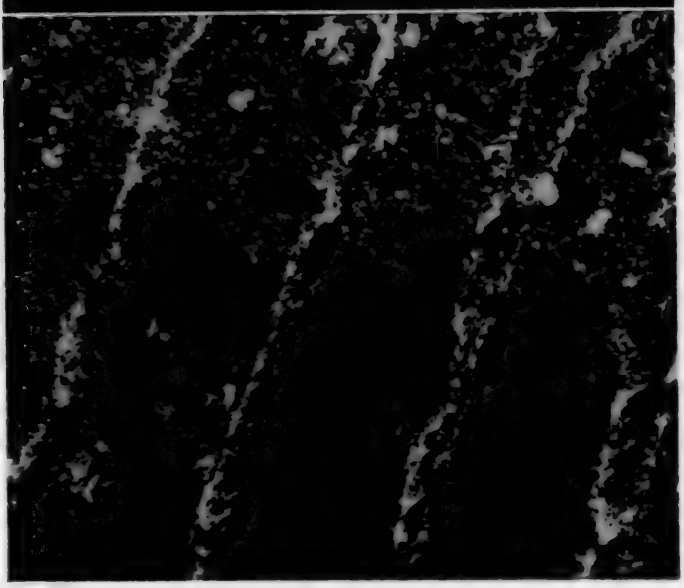

Fig. 5.—Longitudinal section through a mottled enamel (optical micrograph) ×47. Polished with chromium oxide. (Upper left canine of 16-year-old woman.)

Fig. 6.—Longitudinal section through a mottled enamel ×3,800. Polished with chromium oxide. (Upper right central of 16-year-old woman living in an area where the drinking water contains 5.6 ppm fluorine.)

the enamel surface. Part of the surface in Fig. 5 is seen under the electron microscope in Fig. 6. The prismatic structure is seen, and it is evident that the interprismatic part is more readily worn down by the friction (of polishing) than is the rod.

Fig. 7.

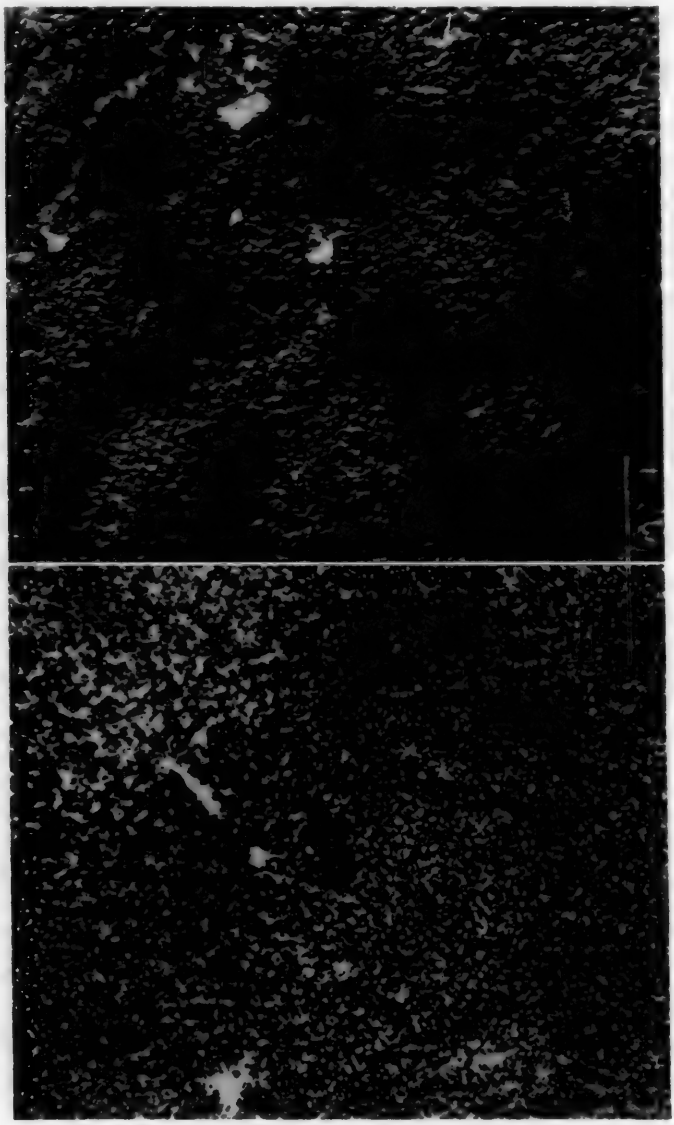

Fig. 8.

Fig. 7.—Surface of a dilacerated tooth ×3,500. (Labial surface of upper right lateral.)
Fig. 8.—Surface of a retention tooth ×3,800.

Dilacerated Tooth.—Two upper incisors with a distinct curvature to their roots were examined. The enamels appeared almost perfect under the optical microscope. Under the electron microscope their surface particles are rough as shown in Fig. 7, but in this figure a small, flat defect is observed at the upper left.

Fig. 9.

Fig. 10

Fig. 9.—Surface of a tooth in dentigerous cyst ×8,700.
Fig. 10.—Surface of carious enamel ×3,200. (Medial surface of lower right second molar.)

Unerupted Tooth.—A completely unerupted tooth of a 52-year-old man was a little smaller than a normal premolar, had a slight root curvature, and a surface which was slightly rough and opaque, but not chalky. Its rough surface is seen in Fig. 8.

Fig. 11.

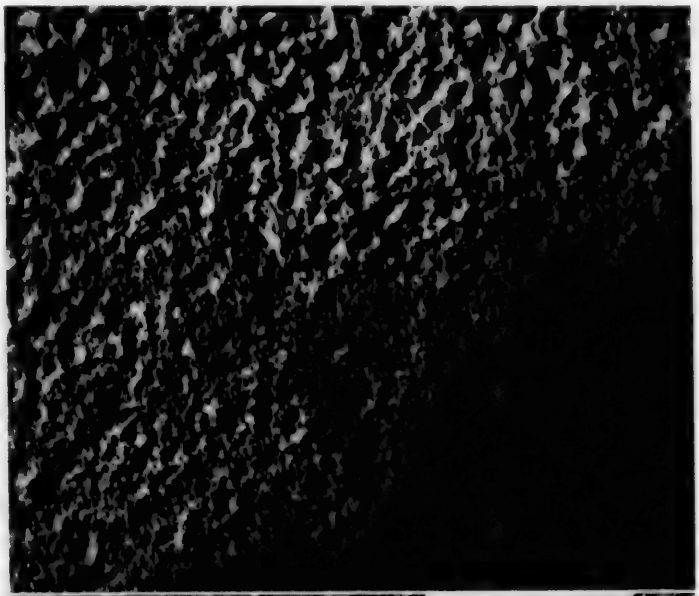

Fig. 12.

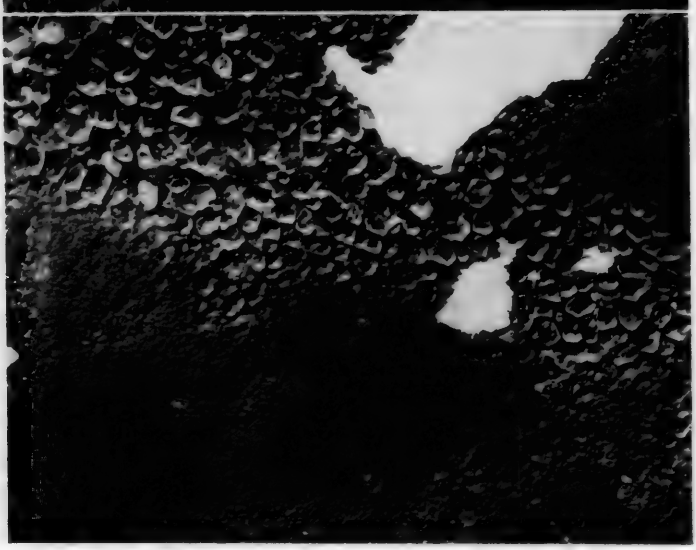

Fig. 11.—Surface of carious enamel (diffuse form) ×4,000. (Buccal surface of lower right third molar.)

Fig. 12.—Carious change noted on a mottled enamel surface ×4,100. (Labial surface of upper left second incisor.)

Tooth Within a Dentigerous Cyst.—Two molars within dentigerous cysts were examined. Visually, these teeth were perfect in development. In Fig. 9, a polygonal rod end and rough particles are seen. In another case, also, surface roughness was noted.

Fig. 13.

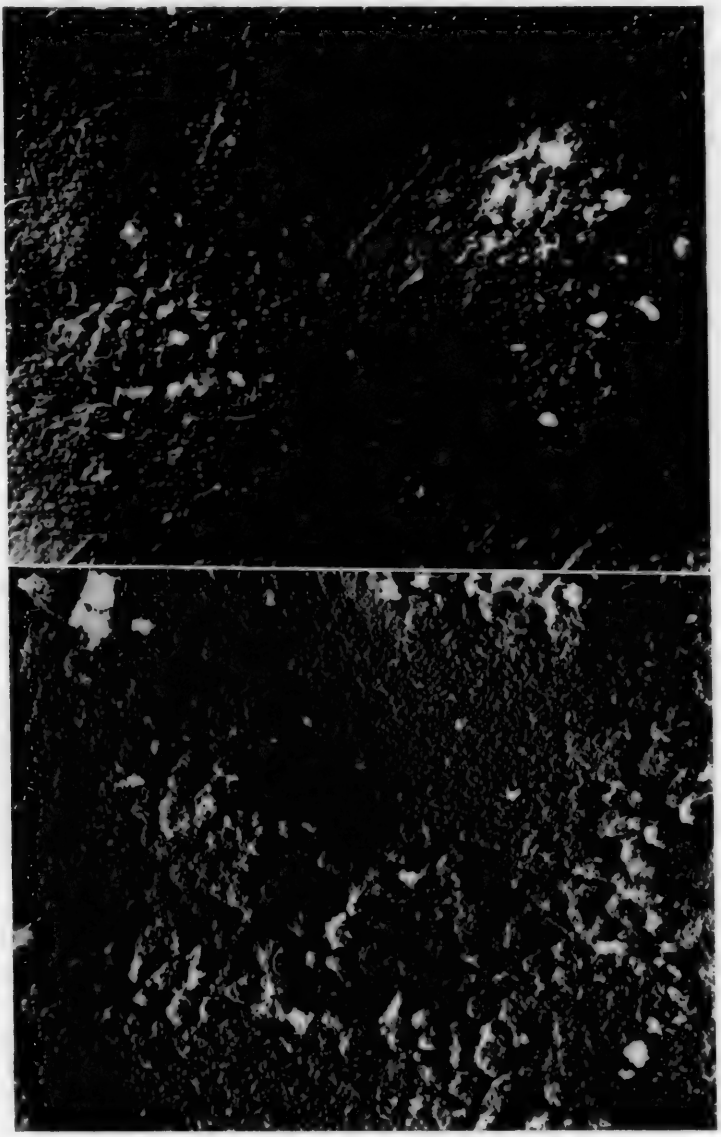

Fig. 14.

Fig. 13.—Surface of an artificially carious enamel ×7,900. (Labial surface of lower right third molar.)

Fig. 14.—Surface of an artificially carious enamel ×3,400. (Buccal surface of upper left third molar of 29-year-old man.)

Hard Odontoma.—Two hard odontomas were examined. The rough particles were seen as well on their enamel surface as on the other abnormal teeth.

Carious Enamel Surfaces.—Previous studies[3] showed that carious changes begin with roughening at the rod end and then formation of grainlike defects

Fig. 15.

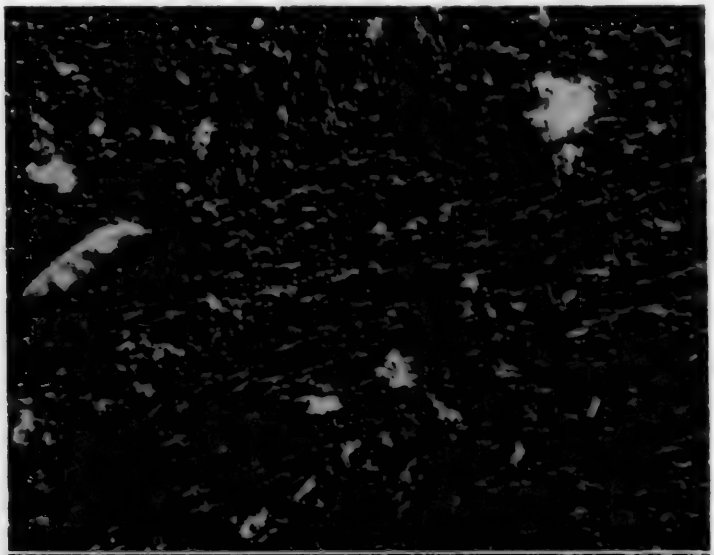

Fig. 16.

Fig. 15.—Surface of an artificially carious enamel ×9,500. (Sectioned surface of an upper right first molar.)

Fig. 16.—Minute particles of the outer surface of normal enamel ×10,000. (Buccal surface of lower right third molar of 20-year-old female.)

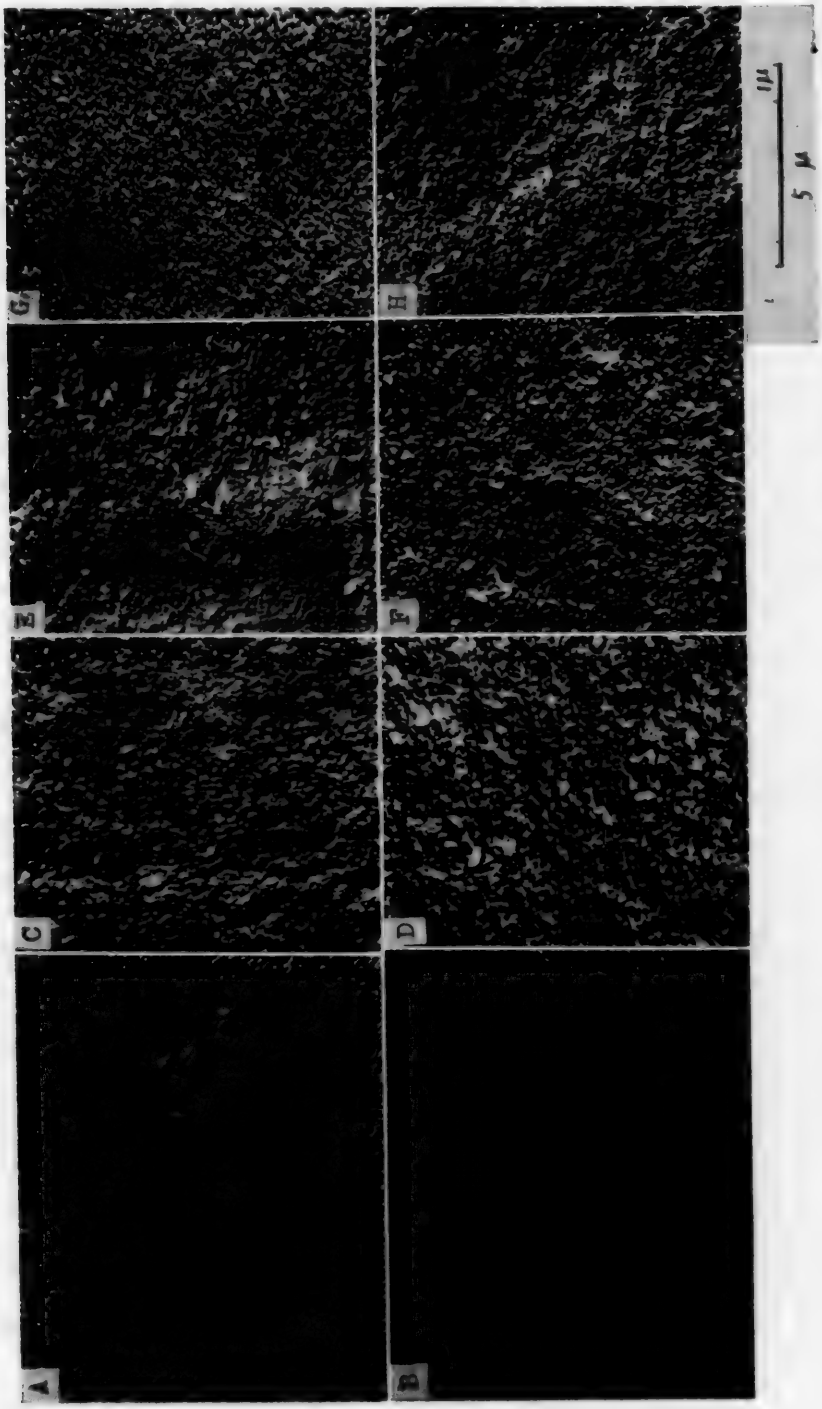

Fig. 17.—Enamel surfaces of normal and abnormal teeth. *A*, *B*, normal enamel; *C*, mottled enamel; *D*, dilacerated tooth; *E*, *F*, hard odontoma; *G*, tooth in dentigerous cyst; *H*, unerupted tooth.

invades the interrod portion; and, after fusion of rod ends, decays the enamel step by step. Fig. 10 shows how changes begin on rod ends, left, and then spread, right.

I regard this as the principal process in caries on smooth surfaces; however, it is not always regular. In some instances (Fig. 11) it cannot be ascertained whether the process started at the rod or interrod portion. In Fig. 12 the carious process appears to be involving enamel in a layerlike defect. This is on an area of mottled enamel.

The same grainlike defect is seen on surfaces subjected to artificial caries (Figs. 13 and 14) produced by a method previously described.[5] The tooth, with a small area showing through a waxed surface, is suspended in a mixture of 1 Gm. of bread and 8 c.c. of saliva at 37° C. for 48 hours. Fig. 14 shows the resemblance to natural caries. In Fig. 15 the polished surface of a tooth is seen after it was subjected first to sodium fluoride and then to the artificial caries process. This change is much different from those produced on the surface.

By contrast, a normal surface of caries-free enamel is seen in Fig. 16.

DISCUSSION

In abnormally formed enamel, rough surface particles of uneven size are observed in shadowed replicas at high manifestation. Because of the resemblance of these surfaces to incompletely calcified enamel,[3] they can be regarded as examples of abnormal formations, although the abnormality may not be seen with the naked eye or under the optical microscope.

While it is still difficult to define the border line between normal and abnormal enamel surfaces, as seen under the electron microscope, if the particles are minute and of even size, the surface may be regarded as normal (Fig. 16). The diameter of the particles shown ranges from 0.05 to 0.15 μ. Fig. 17 shows normal and abnormal enamel surfaces under the same magnifications.

Although in some instances it is difficult to ascertain whether caries starts in the rods or in the interrod portions, in no case could primary involvement of the interrod portion be recognized, but in many cases caries could be seen beginning in rods. In artificial caries it is still not known why changes on the outer surface appear the same as in natural caries but unlike natural caries on polished surfaces.

SUMMARY

Various abnormal enamel surfaces (mottled enamel, dilacerated tooth, unerupted tooth, tooth in dentigerous cyst, hard odontoma) were examined under the electron microscope. The border line between normal and abnormal enamel surfaces is discussed.

The carious changes of natural and artificial foci on enamel surfaces are described.

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STUDIES IN THE REATTACHMENT AND REGENERATION OF THE SUPPORTING STRUCTURES OF THE TEETH

III. Regeneration in Epithelized Pockets

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THE ultimate aim of these investigations is to develop improved clinical methods for promoting regrowth of the supporting structures of teeth lost because of periodontal disease. This is the third paper in a series reporting an investigation of the fundamental factors concerned in the reparative process. Dogs were used as the experimental animals. The first paper[24] dealt with reattachment by regrowth of new cementum after the surgical deepening of the gingival crevice by detachment of the gingiva and removal of a section of alveolar process and periodontal membrane. The second study[25] demonstrated, in addition to reattachment, regeneration of the alveolar process with the establishment of a functional periodontal membrane. The simplified experimental conditions have permitted a study of the manner in which the supporting structure is regrown and of the nature of the reparative process.

The present study deals with an investigation of the same problem, but with the experimental conditions more closely simulating the clinical situation in periodontal disease, i.e., following the creation of infected epithelized pockets.

EARLIER INVESTIGATIONS

Ever since Younger[36] suggested the idea in 1905, periodontists have been fascinated by the possibility of reducing or eliminating a periodontal pocket by reattachment. Periodically. reports of reattachment following treatment have appeared in the literature.[5-7, 12-17, 19, 20, 23, 27, 31-33] The first attempt to support these claims by histologic evidence was made by Box[12] in 1924. He prepared sections of a tooth treated by McCall in 1912 and extracted in 1920. Five years later Box[13] reported a short-term study (1 to 9 days) of 3 cases using the same technique as McCall.

Beckwith, Williams, and Fleming[2] were the first to investigate this problem using laboratory animals. They reported regeneration following the surgical destruction of a section of the supporting structure in rodents, but in an area remote from and not involving the gingival crevice. Later they reported a similar result with cats.[3] Since that time conflicting findings have been reported by a number of investigators, who used various animals and methods.[4, 5, 10, 24, 25, 30, 34, 35]

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Although there is disagreement as to the possibility of inducing reattachment in periodontal pockets, most workers accept the view that once the epithelial attachment has migrated apically there can be no attachment of the connective tissue to the denuded root. Whether or not reattachment may be induced after removal of the pocket epithelium is the controversial issue.

This study was inspired by the clinical impression that reattachment sometimes results from treatment where no attempt has been made to remove the pocket epithelium. The apparent reattachment was observed usually after an interval of 1 to 2 years from the initial treatment. Accordingly, it was planned to simulate as far as possible in dogs the situation one hopes to attain by the conservative treatment of a pocket without curettage of the soft tissues, and then to study the effect of a prolonged healing period of 16 to 18 months. This work was carried out in 15 cases, with healing periods lasting up to 18 months. It was found that, in most cases, there was considerable regeneration of the supporting structures. In one case there was almost complete reconstruction of the suspensory apparatus (bone and periodontal membrane).

How did this regeneration come about? Until we know more of the nature of the physiologic processes concerned in this repair, clinical application will remain a hit-or-miss affair. Accordingly, based on the findings of our previous studies, an attempt has been made to reconstruct the sequence of the changes by which the regeneration progressed.

EXPERIMENTAL PROCEDURE

The experimental animals were 5 dogs with healthy gingival tissues. Only the 4 canine teeth were used. By means of a disc stone, a groove was cut into the tooth approximately 1 mm. from the gingival margin. This groove served as a point of reference from which the level of the gingival sulcus or pocket could be measured. By means of a probe graduated in millimeters, the depth of the gingival sulcus from the groove in the tooth was recorded. A gingival flap was made exposing the alveolar bone. Then a section of bone and periodontal membrane approximately 7 by 6 mm. was removed, exposing the tooth. The measurement from the groove in the tooth to the attachment at the base of the lesion was approximately 10 mm. The exposed root was lightly scaled to remove any remaining soft tissue. The space created by the removal of the alveolar process was then filled with periodontal cement pack,[26] which was allowed to set. After the cement pack hardened, the flap was sutured into place. The cement pack was used to prevent reattachment and to permit the epithelium to cover the pocket wall, creating an epithelized pocket.

Three weeks later the animals were again anesthetized and the pack, if still present, was removed. The pack, when found, was usually loose in the pocket. The measurement from the groove in the tooth to the bottom of the pocket and the amount of gingival recession were recorded. Nothing further was done for 3 months. At the end of this period smear and dark-field examination indicated that all the pockets exhibited flora typical of that usually

encountered in periodontal disease. The measurement from the groove in the tooth to the bottom of the pocket and the amount of recession were again recorded.

The pockets were then packed with periodontal cement pack for 24 hours. Smear and dark-field examination indicated that they were then "clinically sterile."[26] The root surface was scaled.

Nothing further was done for 15 months. The measurement from the groove in the tooth to the bottom of the sulcus or pocket and the amount of gingival recession were again recorded. The animals were sacrificed and blocks were removed for sectioning. Paraffin sections were made after fixation in 10 per cent Formol-saline and decalcification in 5 per cent nitric acid, and were stained with hematoxylin and eosin. The sections from one block were technically unsatisfactory and this block was eliminated, leaving 15 pockets in 4 dogs at the end of the study. One animal had died early in the study.

OBSERVATIONS ON THE LIVING ANIMAL

Before the operative procedure, and at intervals throughout the study, measurements were made from the groove on the tooth to the line of attachment. The pocket depth was also recorded. Thus, by simple calculation the amount of reattachment and recession could be estimated. After the third month the line of attachment was found to be uneven and the measurement recorded was the distance from the groove to the most apical point. Thus sections would often show more reattachment than was recorded in the observations on the living animal.

Table I gives the data on 15 teeth before operation and after at intervals of 3 weeks, 3 months, and 19 to 21 months after the surgical procedure.

TABLE I

OBSERVATIONS IN THE LIVING ANIMAL
MEASUREMENTS IN MILLIMETERS FROM THE GROOVES ON THE TEETH TO THE LINES OF ATTACHMENT AND POCKET DEPTHS BEFORE SURGERY AND AT INTERVALS AFTER SURGERY

	BEFORE SURGERY		AFTER SURGERY		THREE WEEKS AFTER SURGERY		THREE MONTHS AFTER SURGERY		19 TO 21 MONTHS AFTER SURGERY		
TOOTH	ATTACHMENT	GINGIVAL CREVICE	ATTACHMENT	POCKET DEPTH	ATTACHMENT	POCKET DEPTH	ATTACHMENT	POCKET DEPTH	ATTACHMENT	POCKET DEPTH	REATTACHMENT
1	3	2	10	9	11	7	7	5	5.5	2	4.5
2	3	2	11	10	9	6	6	4.5	6	2	5
3	3	2	10.5	9.5	9	7	5	3	6	1	4.5
4	3	2	11	10	12	7.5	11	8	11	6	0
5	3	2	10	9	10	7	8	7	4	2	6
6	3	2	10	9	10	8	8	4	4	2	6
7	3.5	2.5	10	9	10.5	8.5	6.5	5	5	2	5
8	3	2	10	9	10	7	6.5	4	7	3	3
9	3	2	10	8	10	7	6.5	5	6	3	4
10	3	2	10	9	10	6	9	4	8.5	3.5	1.5
11	3	2	10	9	10	6	6	3	5.5	2.5	4.5
12	3	2	9	8	9	5	5	3	4	1	5
13	3	2	9	8	9	5	5	3	3.5	0.5	5.5
14	4	3	11	10	11	7	7	5	5.5	1	5.5
15	3	2	10	9	10	7	7	6	3	1	7

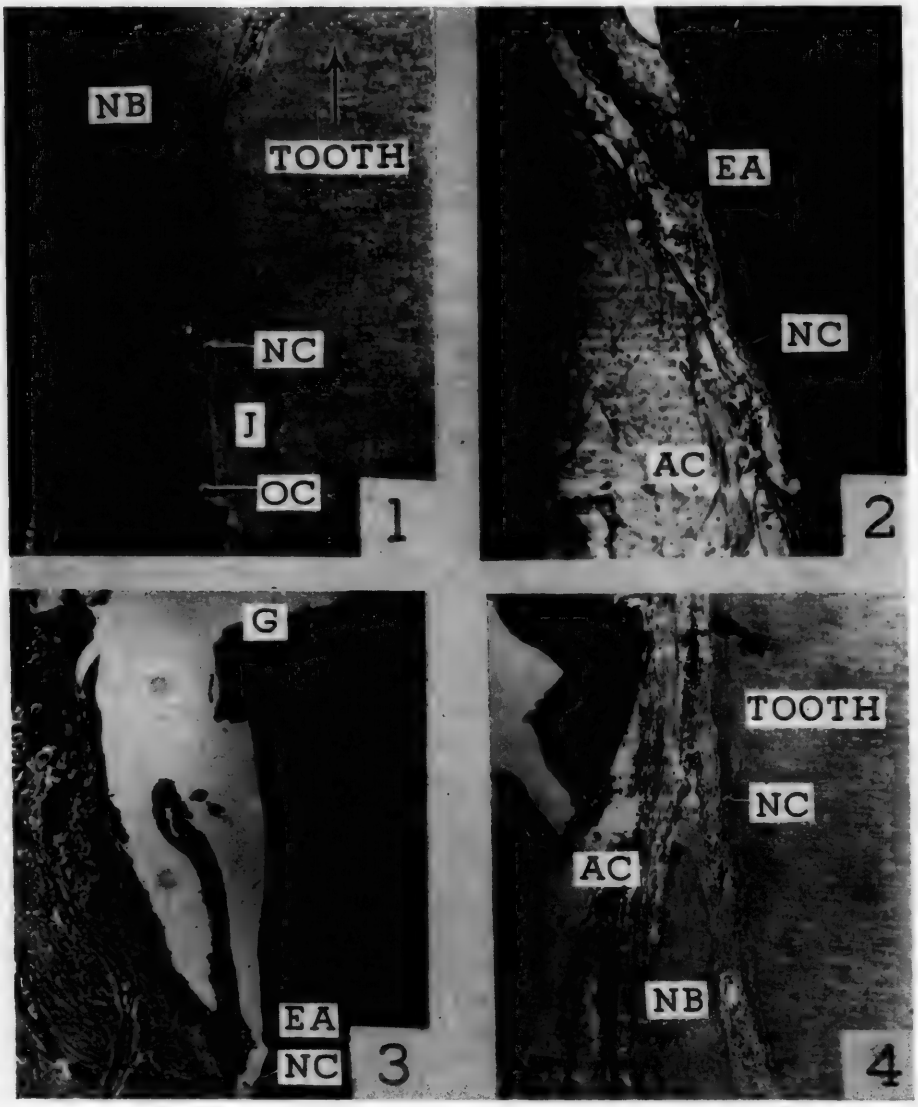
HISTOLOGIC OBSERVATIONS

As was pointed out in Studies 1 and 2, an irregular outline of the root surface indicates an area that had been surgically denuded. This is clearly illustrated in Fig. 1. The regular outline of the undisturbed part of the cementodentinal junction in the area below *J* may be compared with the irregular outline of the denuded section above that point. It follows, then, that any bone, periodontal membrane, or cementum opposite such an irregular root surface must be new tissue—the alveolar bone and periodontal membrane must, obviously, have been removed before the root could have been denuded.

In the instance of Tooth 15 soft tissue was attached by means of new cementum up to the original line of attachment. There was almost complete regeneration of the alveolar bone, and new periodontal membrane was formed. Altogether, there appeared to be almost complete functional and structural regeneration of the lost supporting structure. Fig. 1 is a low-power magnification at the junction of the old and new attachment. It is evident in the reattached area that the original cementum and some of the dentin has disappeared, either at the time of operation or later by resorption. New cementum is present above *J*. The alveolar process has followed the new outline of the root. Fig. 2 shows a fairly normal epithelial attachment. Fig. 3, a low-power view of another section of the same tooth, shows that the epithelial attachment is less than 1½ mm. from the groove in the tooth, since the diameter of the low-power field is approximately 1½ mm. It indicates that complete soft-tissue reattachment has occurred in this section. Fig. 4 is a low-power magnification showing the alveolar crest. In this case there was approximately 6 mm. of alveolar bone replaced, which represented almost complete regeneration. Notice the peculiar appearance of the alveolar crest, which looks as if it had a fibrous cap. This was characteristic of the alveolar crest in this study whenever bone regeneration occurred.

The histologic appearance of the tooth in the reattached area differs from that observed in Study 1 in this series. Fig. 5 is a low-power magnification of a reattached area just below the epithelial attachment. Notice that there has been a continuous resorption of the old cementum and part of the dentin up to the epithelial attachment. Compare this with the irregular type of resorption in Fig. 6, illustrated by a comparable area taken from Study 1.

Fig. 7 is a low-power magnification of the alveolar crest in a tooth where there was 6 mm. of reattachment, but for some reason, as yet unknown, there was little regeneration of alveolar bone. This fact is determined by the position of the alveolar crest, which is apical to the junction of the old and new attachments. The resting line at the alveolar crest also indicates inactivity. In contrast, in Fig. 8, from another tooth, it is evident that the replacement of bone kept pace with the reattachment process. Notice again the appearance of the alveolar crest characteristic of those cases where the alveolar process is being replaced.



Key to abbreviations in illustrations: *J*—Junction of old and new attachments; *G*—Groove in tooth; *EA*—Epithelial attachment; *AC*—Alveolar crest; *NC*—New cementum; *OC*—Original cementum; *OCL*—Osteoclast; *NB*—New bone; *T*—Tooth; *IRL*—Irregular resting line; *RL*—Resting line; *CL*—Cement lines; *OB*—Osteoblasts; *OP*—Original alveolar process; *PM*—Periodontal membrane; *LB*—Lamellar bone; *NLB*—Nonlamellar bone; *CB*—Collagen bundles in cross section.

Fig. 1.—Case 15, low-power magnification of junction of old and new attachments, arrow pointing crownwise.

Fig. 2.—Case 15, low magnification showing well-formed epithelial attachment.

Fig. 3.—Case 15, epithelial attachment approximately 1.5 mm. from groove in tooth; low power.

Fig. 4.—Case 15, low-power magnification of new alveolar crest. Notice fibrous-appearing cap.

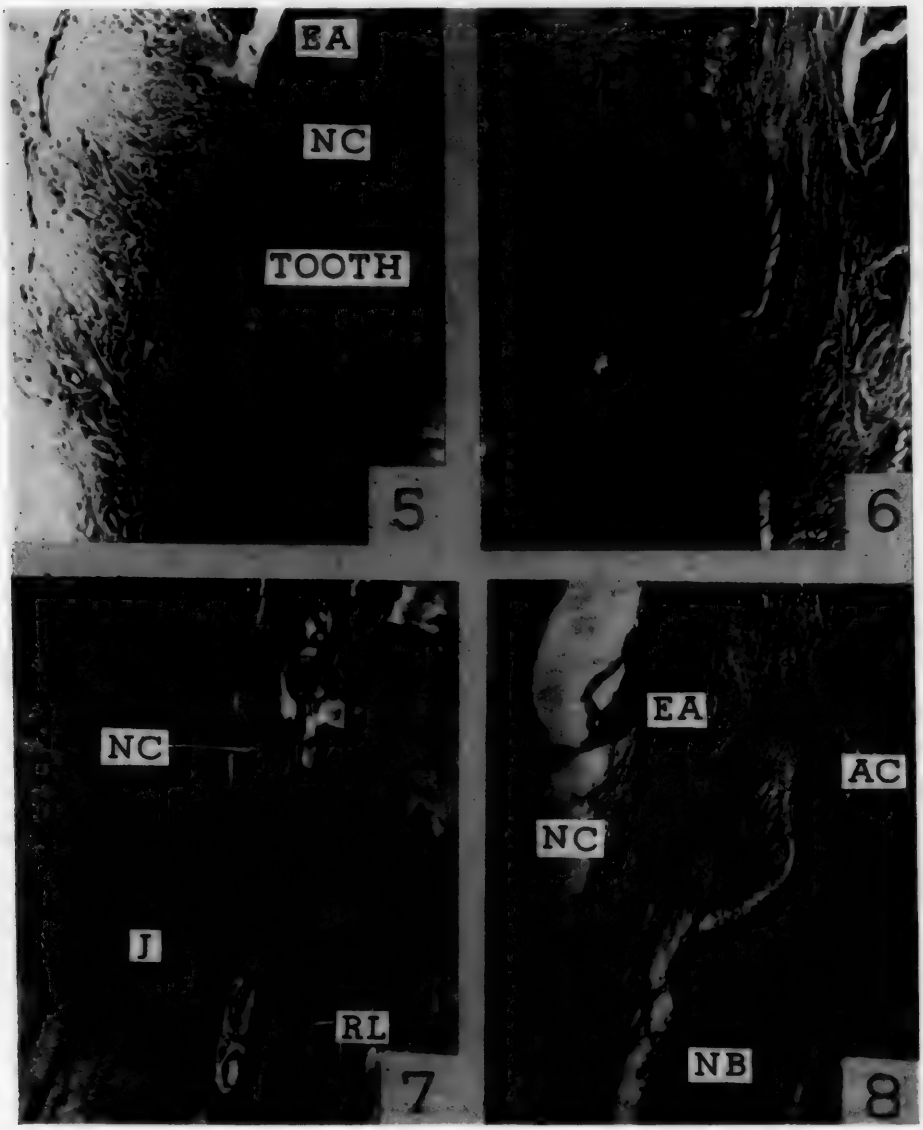


Fig. 5.—Case 15, low-power field just apical to the epithelial attachment. Notice continuous resorption of the dentin.

Fig. 6.—Forty-nine-day specimen. Low-power field just apical to the epithelial attachment (Study 1). Compare interrupted resorption of the dentin with the continuous resorption in Fig. 5.

Fig. 7.—Case 14, 21-month specimen. Alveolar crest still apical to the junction of the old and new attachments, indicating little regeneration of the alveolar process. Notice the resting line at the crest. There was 5.5 mm. of reattachment. Low-power magnification.

Fig. 8.—Case 9, 19-month specimen. Regeneration of the alveolar process keeping pace with the reattachment process. Note characteristic appearance of the alveolar crest. Low-power magnification.

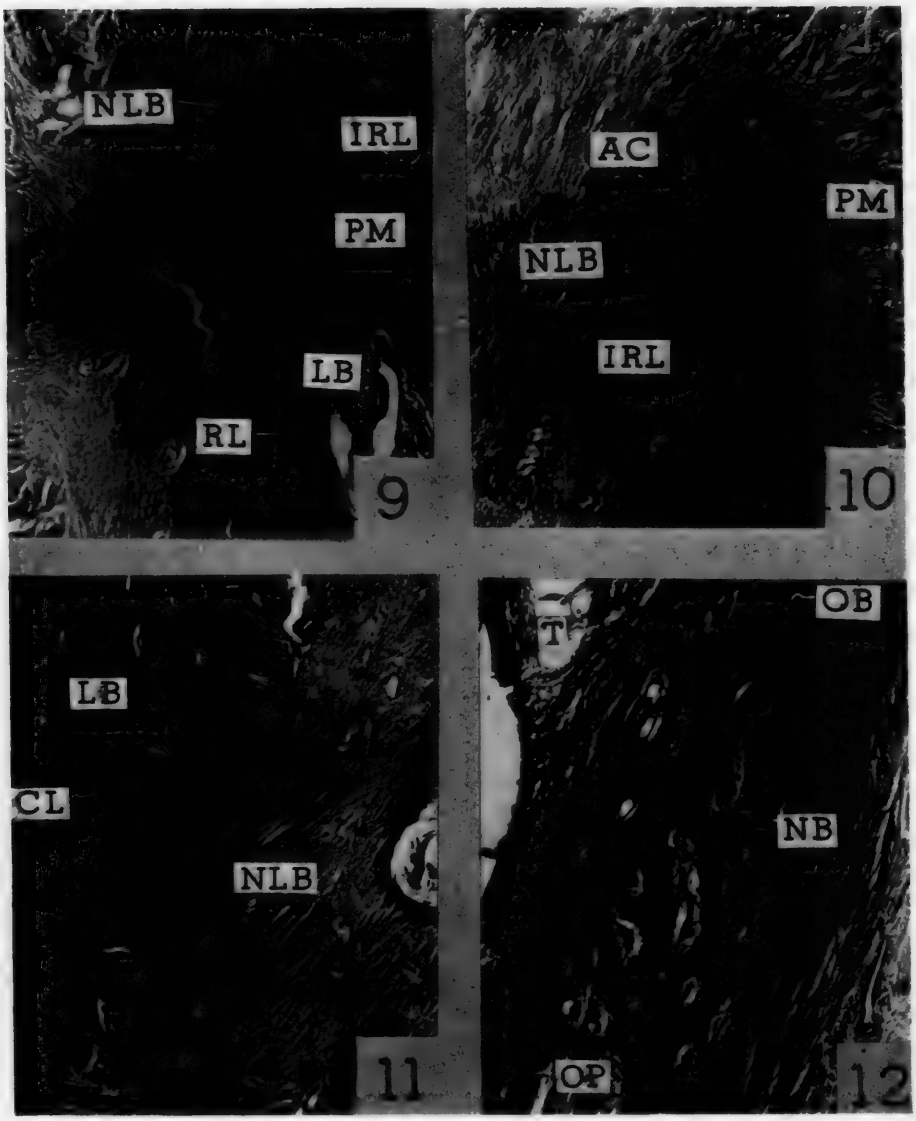


Fig. 9.—Case 7, 19-month specimen. Notice irregular cement lines, *IRL*, in the coarse-fibered bone. Compare with the more regular cement lines, *RL*, in the lamellar bone.

Fig. 10.—Case 7. High-power magnification of Fig. 9. Notice coarse-fibered bone at the crest, and the irregular resting line, *IRL*, in this type of bone.

Fig. 11.—Case 7. High-power field 1 mm. from the margin of the bone at the crest. Mature lamellar bone replacing the coarse-fibered bone.

Fig. 12.—Low-power magnification of new bone, 28-day specimen, where no pack was used (Study 1), showing rapidly growing coarse trabeculae of nonlamellar bone. Notice the differentiation of osteoblasts in advance of the bone. Compare with Fig. 13.

Fig. 9 is a low-power view of the alveolar crest in another tooth. The crest of the bone has an irregular brushlike margin. The irregularity of the advancing border is also suggested when the cement lines (I.R.L.) are compared with more regular lines (R.L.) deeper in the bone. The coarse, fibrillar nature of the bone at the crest and the irregular cement lines can be seen more clearly in Fig. 10, a high-power magnification of the same section. The fibers emerge from the bone more or less at right angles and pass out to mingle with the surrounding connective tissue. In Fig. 11, a high-power view one field away from the margin of the bone at the alveolar crest, it appears that the coarse-fibered bone is being built on and replaced by lamellar bone. Small amounts of this coarse-fibered bone could be discerned throughout the replaced bone in some of the cases. However, in most instances, this type of bone was observed only at the alveolar crest.

Fig. 13.—Twenty-five-day specimen following procedure with cement pack as used in the present study. *A*, Seam of osteoid tissue, *CB*, large collagen bundles cut in cross section; *B*, calcified matrix of nonlamellar bone. Below, at *C*, seam of lamellar osteoid tissue. Medium-power magnification.

Fig. 14.—Low-power magnification of Fig. 13. Osteoclasts resorbing new nonlamellar bone.

Fig. 12 illustrates the replacement of the alveolar process at 28 days, observed under the conditions of Study 1. Coarse trabeculae of a nonlamellar type of bone appear to have grown out into the soft tissue. The osteoblasts differentiate in advance of the bone in the direction of growth. In the new trabeculae the osteocytes are larger, stain more deeply, and are more irregularly distributed than in lamellar bone. The growth process seen in Fig. 12 may be compared with that in Fig. 13 (a medium-power magnification), at 28 days, where the cement pack prevented the rapid cellular replacement in the

space created by the removal of the section of bone and periodontal membrane. In the upper part of Fig. 13, *A*, there appears to be a seam of osteoid tissue. The circular formations in the uncalcified matrix are collagen bundles cut in cross section. The collagen bundles can be seen also in the calcified matrix. The irregular distribution of the osteocytes and the coarse irregularly arranged bundles of fibrils indicate that this is a nonlamellar type of bone. The seam of osteoid tissue at *O* suggests that this nonlamellar bone is being used as a scaffolding for the lamellar bone. Fig. 14, a low-power magnification of the same section, illustrates the presence of osteoclasts, indicating that this new nonlamellar bone already is being resorbed.

DISCUSSION

Table I shows that the average depth of the gingival sulci as measured by the graduated probe was approximately 2 mm. before the operative procedure. Excluding teeth 4 and 12 (considered failures), it is interesting to note that at the end of the study the average depth of the gingival sulci was again 2 mm. In most instances this result was achieved by both reattachment and recession. This observation suggests that if the normal arrangement of the tissues with a shallow sulcus is disturbed there is a tendency for the tissues themselves to reassume this desirable structural arrangement. This is of interest in view of the controversy regarding the necessity for complete elimination of the pocket in treatment.

The measurement from the groove in the tooth to the line of attachment shown in Table I "at 3 weeks after surgery" must not be taken as accurate. The procedure was to insert the probe until a definite resistance was encountered, and then to record the measurement from the groove in the tooth. Short-term studies to investigate early changes indicate that even if some reattachment had occurred, the new attachment would not yet have been strong enough to offer a detectable resistance to the probe. The probe would thus be inserted until the alveolar bone at the base of the lesion was encountered.

It appears from the evidence that regeneration of supporting structure may occur without the removal of the pocket epithelium. For the reasons given in the first part of this paper, an hypothesis is advanced to describe the manner in which the regeneration progressed. The reattachment process will be dealt with first.

Fig. 15 is a diagrammatic representation of the experimental pocket. The cells at *X* proliferate, displacing the epithelium crownwise as illustrated in Fig. 15, *B*. In Studies 1 and 2 it was shown that cells proliferating from the bone margin have osteogenic potentiality, and that under suitable environmental conditions those cells that come in contact with the tooth differentiate into cementoblasts in approximately 16 days. Also, tooth structure exposed to these cells is resorbed until the differentiation takes place. Fig. 15, *C* represents the resorption before apposition begins. The cementoblasts form new cementum attaching the connective tissue fibers to the tooth (Fig. 15, *D*).

As this repair proceeds slowly, the resulting histologic picture should be as shown in Fig. 15, *E*. A comparison of Fig. 15, *E* and Fig. 5 suggests that the hypothesis is consistent with what actually occurred. But if the intermittent type of resorption shown in Fig. 6 were encountered, the hypothesis would

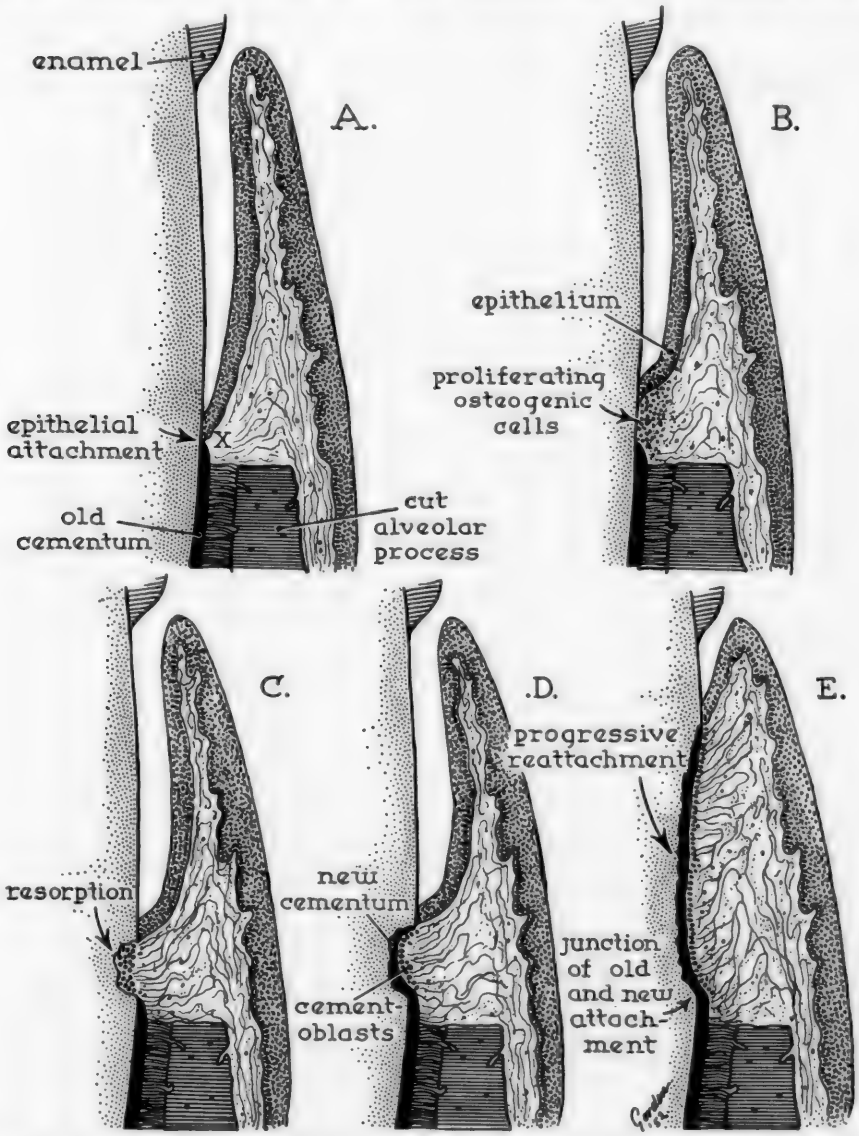


Fig. 15.—Schematic representation of the steps in reattachment according to our hypothesis. (a) Pocket. (b) Cells at *X* have proliferated displacing the epithelium crownwise. (c) Tooth exposed to new cells is resorbed until differentiation of cementoblasts takes place. (d) Cementoblasts have differentiated and begun apposition of new cementum. (e) Appearance of reattached area after the reparative process has progressed for some time, showing continuous resorption of tooth.

be proved to be wrong. A careful examination of all the sections was made, and in no case was the intermittent type of resorption encountered in this study.

The precise manner in which repair of bone occurs has been a matter of dispute for the past hundred years. Thus, the mechanism by which replacement of the alveolar process is brought about cannot be described with any degree of certainty. As different opinions are held about certain features of osteogenesis, there is as yet no generally accepted nomenclature for all phases of bone repair.

The terms "nonlamellar" (woven) and "lamellar" bone, as defined by Baker,[1] will be used in this paper. Lamellar bone presents no particular difficulty, as this term is used by most authors with the same meaning. In the case of nonlamellar (woven) bone, there is no standard nomenclature in the English literature; it has been called by various writers "coarse-fibered bone," "fiber bone," and "immature bone."

Baker described 2 types of woven bone. The first, like lamellar bone, is formed by the osteoblasts. According to Baker, any bone tissue formed rapidly under pathologic conditions is of this first type. Eventually it is replaced by lamellar bone. An example is the woven bone forming the "provisional callus" of a fracture being replaced by the lamellar bone of the "definitive callus." The replacement of bone in Study 1 appears to have proceeded by a method similar to that of a healing fracture, the regenerating alveolar process being first laid down as woven bone (Fig. 12) which is gradually replaced by lamellar bone. The stimulating effects of autogenous grafts of bone and dentin in the rebuilding of the alveolar process are described in Study 2.

In the present study the manner in which the alveolar process has regenerated appears to differ in some respects from that observed in Study 1. While the new bone was laid down as woven bone and replaced by lamellar bone, the woven bone resembled histologically the second type described by Baker (formed, he proposed, not by the activity of osteoblasts but by a metaplasia of already existing connective tissue). The woven bone was not laid down in the form of irregular coarse trabeculae, as in Study 1, but more in the manner in which lamellar bone is formed.[21] However, this woven bone is not necessarily formed by metaplasia of connective tissue. The morphologic differences are possibly due to the conditions under which osteogenesis occurred. The conditions in Studies 1 and 2 were such that the space created by the removal of the section of alveolar process was rapidly filled with a largely cellular tissue originating, we believe, in the bony margins of the wound. It appears that these cells were able to differentiate to osteoblasts well in advance of the regenerating bone (Fig. 12). Under such conditions the formation of bone is quite rapid. In the present study, the growing bone had to advance through an already differentiated, mature fibrous connective tissue. No differentiation could take place except at the advancing bone surface. The bone formation was therefore a slow process. One would expect that under these conditions cellular and intercellular elements of the connective tissue would be included

in the bony matrix, giving it the characteristics of Baker's second type (Figs. 9 and 10). If this hypothesis is correct, then both types of woven bone (Figs. 10 and 12) were formed by the activity of osteoblasts, the morphologic differences being due to the different conditions under which osteogenesis occurred.

The tooth shown in Fig. 9 is interesting in that the presence of woven bone at the tip suggests that the regeneration of the bone is still going on after 19 months. In the tooth shown in Fig. 7, where there was no regeneration, the alveolar process consists of lamellar bone only. The presence of cement lines in the woven bone in Fig. 9 indicates that resting periods occurred in the reparative process.

Fig. 7 suggests that considerable reattachment may be secured without regeneration of the alveolar process. Such reattachment would not be discernible in the radiograph. Thus, if it is evident clinically that reattachment has been secured, without regeneration of bone appearing in the radiograph, it could indicate that an etiological factor concerned with bone loss, such as an occlusal factor, is still operating.

Smith,[31, 32] Stillman,[33] McCall,[27] Box,[13] Björndahl,[7] Beube,[5] Goldman,[19, 20] and Carranza[17] have presented radiologic evidence that indicates, in their opinion, regeneration of the supporting structures. This evidence has long been questioned.[8, 29, 30] Assuming that regeneration of supporting structures did take place in their cases, it is interesting and possibly of value to speculate on the manner in which it occurred. The interval between the preoperative and postoperative radiographs (over 1 year in most cases) suggests that regeneration occurred by the slower process described in this paper, rather than by the more rapid processes of Studies 1 and 2, where reattachment was achieved in 20 days and bone formation in 50 days. If such is the case, the removal of the pocket epithelium with the formation and protection of a blood clot in treatment had little bearing on their results.

It has been known for a long time that alveolar process may be regenerated if no detachment of the soft tissues has taken place.[30] The process of regeneration has never been described, to our knowledge. It seems probable that the bone is regenerated in the manner described in this study rather than as described in Studies 1 and 2. Further obstacles have to be overcome before regrowth of the supporting structures (as in Studies 1 and 2) can be achieved from epithelized pockets.

CONCLUSIONS

Reattachment of connective tissue by means of new cementum was secured in dogs following the production of epithelized infected periodontal pockets.

Exposure of the denuded root surface to the oral fluids did not prevent connective tissue reattachment.

A considerable regeneration of the alveolar process may also take place.

Reattachment may occur with or without regeneration of the alveolar bone.

Regeneration of the alveolar bone takes place by the formation of woven bone, which is built on and replaced by lamellar bone.

Regeneration of supporting structure may occur without the removal of the pocket epithelium and the formation and organization of a blood clot.

The epithelial attachment may move crownwise as well as rootwise, depending on what is occurring in the underlying tissue.

An hypothesis is advanced to explain how regeneration of the supporting structure of the teeth may occur in the experimental animal after the production of epithelized pockets.

The histologic sections were prepared by Mr. W. Wilson; the photomicrographs by Dr. W. Stanley Hartroft.

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EVANSTON DENTAL CARIES STUDY

XII. A Survey of Lactobacillus Counts With Reference to Untreated Carious Surfaces Before and After Exposure to Fluoridated Water

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MANY investigators have brought attention to the relationship between the number of lactobacilli present in the saliva and dental caries rates.[1-5] Recently, considerable interest has become evident as to what possible influence the addition of a fluoride to a communal water supply may have on lactobacillus counts. This question arises since a reduction in dental caries has been shown after fluorine has been added to a communal water supply.[6-9]

It is the purpose of this report to attempt to draw some conclusions from the data gathered in the Evanston Dental Caries Study regarding the relation of lactobacillus counts to dental caries prevalence rates. The study was begun in 1946 and the base-line data were collected during that year.[10] The students in the control area (Oak Park, Illinois) were examined in 1947. Sodium fluoride was added to the Evanston water in February, 1947. To date, about 23,000 children have been examined and approximately 10 per cent of these have contributed saliva specimens for lactobacillus study.

REVIEW OF LITERATURE

In a preliminary report on the relationship of increased fluoride content of a communal water supply in an area in which the water was changed from a source containing 0.1 ppm F to a new source which contained 1.7 ppm F (naturally) Arnold, Dean, and Elvove[11] concluded, "There is no indication according to the percentage distribution that the increased fluoride content of the water supply has as yet influenced the *Lactobacillus acidophilus* counts." They further summarized that the relatively unchanged lactobacillus counts in the designated groups might possibly have been due to dental caries activity in lesions which had started prior to the change of water and which were still active. They estimated that a period of at least 2 years would elapse before any change in the caries rate would be noticeable.

Dean,[12] in the Galesburg-Quincy, Illinois, study, reported, that "the dental caries prevalence in 306 children examined at Quincy was well over three times as high as that observed in the 319 children examined at Galesburg." He also reported, "Bacteriologically, the epidemiological aspects of oral *Lactobacillus acidophilus* in saliva closely reflected the observed differences in caries prevalence rates between the two cities." It was shown in the Quincy examinations that there were approximately 5 times as many children in the 30,000 lactobacilli per milliliter saliva count group as in the Galesburg examinations.

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Davies[13] indicated a shift of about "5 per cent of the cases from the higher count groups (those over 1,000 lactobacilli per ml.) to the lower groups, (those under 1,000 per ml.) in Evanston," after 1 year of fluoridation. Finn and Ast[14] reported that of 244 cases studied in Newburgh, New York, before fluoridation, "63.5 per cent of them had lactobacillus counts of 20,000 or over while 11.9 per cent had counts of less than 100." In 1947, approximately 22 months after fluoridation of the Newburgh water supply, saliva samples from 421 children were examined and "only 47.3 per cent had high lactobacillus counts while 20 per cent had counts of less than 100." In the control area, tests carried out in 1946 and 1947 showed "about 54 per cent with high counts and 16 per cent with low counts."

In studying the relation of caries activity to lactobacillus counts and types, and the fluoride content of drinking water in Boulder and Denver, Colorado, Clapper, Downs, and Heatherman[15] found a greater per cent of the children in the Boulder area (fluorine free) "having lactobacillus counts of over 10,000 per ml., and a smaller per cent having counts between 50 and 9,999 per ml." than in Denver F = 1.0). However, the same percentage in each group had negative counts. The investigators stated that the incidence of caries was higher in children who had high lactobacillus counts than in those with low counts.

Suggestive evidence has also been presented by Clapper, Downs, and Heatherman[16] that children drinking water containing 1 ppm F and who had large numbers of rhamnose-fermenting lactobacillus in their saliva were likely to have increased dental caries in the near future. They noted that when there was no fluoride in the water, nearly all the children had increased caries, regardless of numbers and types of lactobacillus. These authors concluded that finding large numbers of lactobacillus of any type in the saliva was usually associated with a rise in the number of decayed surfaces, but that fluorine in the water (1 ppm) appeared to have no influence on the type of lactobacillus colony established.

METHOD

Collection of Saliva Samples.—Saliva samples were collected at 10 A.M. every Tuesday during the school year from children of 2 age groups, 6 to 8- and 12 to 14-year-old children. The children were not screened in any way except that only continuous residence students were allowed to contribute samples. A wax pellet was used to stimulate the flow of saliva, and the samples were collected before dental examinations were made or radiographs were taken.

Medium and Plating.—A medium of tomato juice, agar, yeast extract, peptonized milk, and peptone, adjusted to a pH of 5.0 to 5.2, was used. A Kahn shaker was employed to agitate the saliva. However, Permar, Kitchen, and Robinson,[17] in using a hand shaker, a Kahn tube shaker, and 3 types of beaters as methods of mixing original specimens to ensure a better distribution of organisms, found that none of the methods insured uniformity of counts. For purpose of comparison and as a precaution against ruling out a saliva sample due to an overcrowded plate, 4 plates were inoculated from every specimen.

Two plates were inoculated with 0.1 ml. of a 1 to 10 dilution, and 2 with a 0.1 ml. of a 1 to 100 dilution. Approximately 0.4 to 0.6 ml. of sterile normal saline solution was used on each plate to ensure a more even distribution of the inoculum. The average of the two 1 to 10 dilution plates was used as a final count unless the growth was too thick to allow accurate counting, in which case the 1 to 100 dilution plates were used. Although Permar, Kitchen, and Robinson[17] found a wide variation in colony count in plates made from single samples of saliva, it was necessary for us to rely entirely upon 1 sample from each individual.

Incubation and Counting.—Plates were incubated for 72 hours at 37° C. A low-power binocular microscope was used to study colony morphology and also to make the counts. Stained cellular preparations were made of each type of colony and all types were classified microscopically according to Hadley and Bunting.[18] The final counts comprised the total of all colonies identified as lactobacillus. No antigenic or fermentative differences were studied.

The cases were grouped according to the lactobacillus colony count. Cases with counts ranging from 0 to 9,999 comprised the low lactobacillus division; counts in the range of 10,000 to 49,999, the moderate lactobacillus division; counts of 50,000 and over, the high lactobacillus division. Negative lactobacillus cases, which are included in the low lactobacillus division, were separated and various tabulations were made on them.

TABLE I

NUMBER OF CASES, UNTREATED CARIOUS SURFACES, AND LACTOBACILLUS-NEGATIVE CASES

YEAR	CASES	NUMBER OF CARIOUS SURFACES	CASES WITH NO CARIOUS SURFACES	NO. L/B NEG. CASES	NEG. L/B CASES WITH NO CARIOUS SURFACES	NO. UNTREATED CARIOUS SURFACES OF L/B NEG. CASES
6- to 8-year-olds						
Low L/B div. (L. per ml. 0-9999)						
		Range				
1946	96	695 (0-35)	12	42	5	193
1947*	47	257 (0-61)	10	21	8	115
1948	178	1,285 (0-41)	44	126	37	462
1950	258	1,476 (0-36)	50	147	38	626
1951	204	1,290 (0-22)	30	130	25	661
1953	165	859 (0-27)	35	95	25	425
Moderate L/B div. (L. per ml. 10,000-49,999)						
1946	31	437 (2-30)	0			
1947*	17	257 (0-41)	10			
1948	66	678 (1-35)	0			
1950	93	1,025 (0-31)	3			
1951	96	935 (0-48)	3			
1953	67	589 (1-31)	3			
High L/B div. (L. per ml. 50,000 and over)						
1946	18	191 (0-47)	1			
1947*	10	107 (0-26)	1			
1948	36	374 (0-38)	1			
1950	14	202 (5-34)	0			
1951	17	235 (3-67)	0			
1953	12	128 (4-24)	0			

*Control.

Statistical Evaluation.—The differences in rates in Table III and the shifts in the cases of the low, moderate, and high lactobacillus division in Table VII were evaluated statistically. Differences considered significant are so indicated. All basic figures are shown in Tables I and II.

TABLE II

NUMBER OF CASES, UNTREATED CARIOUS SURFACES, AND LACTOBACILLUS-NEGATIVE CASES

YEAR	CASES	NUMBER OF CARIOUS SURFACES	CASES WITH NO CARIOUS SURFACES	NO. L/B NEG. CASES	NEG. L/B CASES WITH NO CARIOUS SURFACES	NO. UNTREATED CARIOUS SURFACES OF L/B NEG. CASES
12- to 14-year-olds						
Low L/B div. (L. per ml. 0-9999)						
		Range				
1946	138	1,182 (0-58)	7	54	3	294
1947*	50	368 (0-26)	10	14	4	85
1949	120	1,061 (0-57)	9	52	4	388
1952	129	912 (0-35)	14	55	9	353
Moderate L/B div. (L. per ml. 10,000-49,999)						
1946	67	852 (0-35)	3			
1947*	20	252 (3-37)	0			
1949	79	935 (0-52)	3			
1952	132	1,356 (0-44)	7			
High L/B div. (L. per ml. 50,000 and over)						
1946	19	315 (2-43)	0			
1947*	22	311 (5-27)	0			
1949	18	293 (6-51)	0			
1952	16	164 (3-19)	0			

*Control.

FINDINGS

Considering all cases in each lactobacillus count division, with no regard to year of examination or time of exposure to fluoridated water, the cases in the low lactobacillus division are accompanied by a correspondingly low untreated caries prevalence rate (Table III). The moderate and high lactobacillus division cases are accompanied by a greater untreated caries prevalence rate. This appears to correspond to the findings of Jay and Arnold,[19] and Clapper, Down, and Heatherman[15]; i.e., that a low lactobacillus rate is usually accompanied by a correspondingly low caries prevalence rate, and that the incidence of caries was higher in children who had high lactobacillus counts than in those with low counts.

Considering the untreated carious surface rate by year, it will be noted (Tables IV and V) that the cases in the low, moderate, and high lactobacillus divisions also reflect a corresponding low, moderate, and high untreated carious surface rate in 1948, 1949, 1950, 1951, and 1953. In the younger age children (Table IV) the findings in the years 1946 and 1947 (control) did not accurately fit this pattern, as the untreated carious surface rate in the moderate lactobacillus division was higher than the rate found in the high lactobacillus division. It is also confusing to observe that the cases in the high lactobacillus division in

1950 and 1951 had a higher untreated carious surface rate than those cases in the high lactobacillus division of 1946. The 1946 and 1953 rates in the same division differed only slightly.

TABLE III

NUMBER OF CASES AND UNTREATED CARIOUS SURFACES IN EACH LACTOBACILLUS COUNT DIVISION (1946-1953)

LACTOBACILLUS COUNT DIVISIONS	CASES	UNTREATED CARIOUS SURFACES	UNTREATED CARIOUS SURFACE* RATE PER HUNDRED CHILDREN
Low L/B div.			
6- to 8-year-olds	948	5,862	618.35
12- to 14-year-olds	437	3,523	806.17
Moderate L/B div.			
6- to 8-year-olds	370	3,921	1,059.74
12- to 14-year-olds	298	3,395	1,139.26
High L/B div.			
6- to 8-year-olds	107	1,237	1,156.07
12- to 14-year-olds	75	1,083	1,444.00
Total	2,235	19,021	

*Differences between caries rates of children in the high, moderate, and low divisions are significant.

TABLE IV

UNTREATED CARIOUS SURFACE RATE FOR CASES IN EACH LACTOBACILLUS COUNT DIVISION

YEAR	CASES	EXPOSURE TO NaF (MONTHS)	UNTREATED CARIOUS SURFACE RATE PER HUNDRED CHILDREN	PER CENT DIFFERENCES I INCREASE R REDUCTION
6- to 8-year-olds				
Low L/B div. (L. per ml. 0-9999)				
1946	96		723.95	
1947*	47		546.80	
1948	178	12-22 mo.	721.91	
1950	258	35-46 mo.	572.09	
1951	204	47-58 mo.	632.35	
1953	165	71-82 mo.	520.60	R 28.08
Moderate L/B div. (L. per ml. 10,000-49,999)				
1946	31		1,409.67	
1947*	17		1,305.88	
1948	66	12-22 mo.	1,027.27	
1950	93	35-46 mo.	1,102.04	
1951	96	47-58 mo.	973.95	
1953	67	71-82 mo.	879.10	R 37.63
High L/B div. (L. per ml. 50,000 and over)				
1946	18		1,061.11	
1947*	10		1,070.00	
1948	36	12-22 mo.	1,038.88	
1950	14	35-46 mo.	1,442.85	
1951	17	47-58 mo.	1,382.34	
1953	12	71-82 mo.	1 066.66	R 0.52

*Control.

The cases in each lactobacillus count division of the older age group indicated reductions in untreated carious surfaces when comparing the 1952 examinations to the 1946 examinations (Table V). However, the most consistent reduction was found in the untreated carious surface rate in the moderate lactobacillus division when comparing the rates of 1949 and 1952 to that of 1946. In the high lactobacillus division a slight reduction is found in the untreated

carious surface rate when comparing the rates of 1949 to those of 1946. However, when the 1952 rate is compared to that of 1946, a considerable reduction is observed. The number of cases in the high lactobacillus division in 1946, 1949, and 1952 were small but comparable, 19, 18, and 16, respectively. It is also observed that the untreated carious surface rate in the high lactobacillus division in 1952 was slightly less than the untreated carious surface rate of the moderate lactobacillus division of the same year.

TABLE V

UNTREATED CARIOUS SURFACE RATE FOR CASES IN EACH LACTOBACILLUS COUNT DIVISION (1946-1952), NaF ADDED FEBRUARY, 1947

YEAR	CASES	EXPOSURE TO NaF (MONTHS)	UNTREATED CARIOUS SURFACE RATE PER HUNDRED CHILDREN	PER CENT DIFFERENCES I INCREASE R REDUCTION
12- to 14-year-olds				
Low L/B div. (L. per ml. 0-9999)				
1946	138		856.52	
1947*	50		736.00	
1949	120	23-34 mo.	875.66	
1952	129	59-70 mo.	706.20	R 17.55
Moderate L/B div. (L. per ml. 10,000-49,999)				
1946	67		1,271.64	
1947*	20		1,260.00	
1949	79	23-34 mo.	1,183.54	
1952	132	59-70 mo.	1,027.27	R 19.21
High L/B div. (L. per ml. 50,000 and over)				
1946	19		1,657.89	
1947*	22		1,413.63	
1949	18	23-34 mo.	1,627.77	
1952	16	59-70 mo.	1,025.00	R 38.17

*Control.

TABLE VI

PERCENTAGE OF LOW DIVISION LACTOBACILLUS CASES WITH NEGATIVE LACTOBACILLUS COUNTS

	1946	1947*	1948	1949	1950	1951	1952	1953
6- to 8-year-olds								
Per cent of low L/B div. cases with neg. L/B counts	43.75	44.68	70.78		56.97	63.23		57.57
Per cent of L/B neg. cases with no carious surfaces	11.90	38.09	29.36		25.85	19.23		26.31
Carious surf. rate of L/B neg. cases per 100 children	459.52	547.61	366.66		425.85	508.46		447.36
12- to 14-year-olds								
Per cent of low L/B div. cases with neg. L/B counts	39.13	28.00		43.33			42.63	
Per cent of L/B neg. cases with no carious surfaces	5.55	28.57		7.69			16.36	
Carious surf. rate of L/B neg. cases rate per 100 children	544.44	607.14		746.15			641.83	

*Control.

The percentage of lactobacillus negative cases of the low lactobacillus division increased for both age groups when comparing the results obtained in

1952 and 1953 with those of 1946 (Table VI). The percentage of all lactobacillus-negative cases with no carious surfaces also increased for both age groups; however, the increase was slightly more consistent in the older group than in the younger age group. In both age groups the number of untreated carious surfaces of lactobacillus-negative cases (rate per hundred children) appeared to fluctuate from 1946 through 1952 and 1953.

TABLE VII

DISTRIBUTION OF CASES IN LOW, MODERATE, AND HIGH LACTOBACILLUS COUNT DIVISIONS (1946-1953)

	LACTOBACILLUS COUNT DIVISIONS		
	LOW	MODERATE	HIGH
6- to 8-year-olds			
1946	66.13%	21.37%	12.50%
1947*	63.51%	22.97%	13.52%
1948	63.57%	23.57%	12.96%
1950	70.68%	25.45%	3.97%
1951	64.35%	30.22%	5.43%
1953	63.52%	27.45%	9.03%†
12- to 14-year-olds			
1946	61.60%	29.91%	4.49%
1947*	54.34%	21.73%	23.93%
1949	55.29%	36.40%	8.31%
1952	46.57%	47.65%‡	5.78%

*Control.
†The difference between the 1953 rate and the 1946 rate is significant.
‡This increase from the 1946 rate is significant

There was no noticeable change in the percentage of all cases found in the low lactobacillus division in the 6 to 8-year-old children from 1946 through 1953 (Table VII). However, the percentage of cases in the moderate lactobacillus division increased steadily up to 1953, when it dropped slightly. This increase was apparently at the expense of the cases in the high lactobacillus division, as there was a reduction in this division from 12.50 per cent in 1946 to 9.03 per cent in 1953.

For the older group, the percentage of all cases found in the low lactobacillus division dropped steadily from 1946 through 1949 and 1952. In the moderate lactobacillus division the percentage of cases increased consistently during the same years. However, in the high lactobacillus division, the percentage of cases rose in 1949, and then declined slightly in 1952 to a figure higher than the 1946 percentage figure. Apparently the increase found in the moderate lactobacillus division was obtained from the low lactobacillus division, while the change in the high group was one that would probably occur from year to year.

DISCUSSION

In both age groups there were reductions in the untreated carious surface rates. Also, there were increases in the percentage of lactobacillus negative cases of both age groups, which indicates a reduction of lactobacillus activity. Referring specifically to the younger age group, there was no appreciable change in the percentage of lactobacillus cases in the low lactobacillus division from 1946 through 1953. There was an increase in the cases in the moderate

lactobacillus division. This increase was apparently from the high lactobacillus division as there was a reduction in the cases found in this division. Again this indicates a decrease of lactobacillus activity.

The older age group, with a reduction in untreated dental carious surface rates in all 3 lactobacillus count divisions, had an increase in the number of negative lactobacillus cases. This would indicate a retardation of lactobacillus activity. However, this may be misleading. Considering the fact that a decrease was noted in the cases making up the low lactobacillus division and an increase noted in the cases making up the next higher division, the moderate division, while the cases of the high lactobacillus division remained about the same, an increase in lactobacillus activity is suggested. As the untreated carious surface rate was less in view of an apparent increase in lactobacillus activity, the inference is made that the teeth after fluoridation may be rendered more resistant to etching. The lactobacillus, even though numerous, could be considered less harmful to the teeth. It is also cited that the saliva of these children could be associated with species other than *L. casei* and *L. plantarum*. It was inferred by Clapper, Downs, and Heatherman[16] that children with large numbers of rhamnose-fermenting lactobacillus in their saliva, and drinking water with 1 ppm fluorine, would in the near future, have an increase in dental caries activity. Tilden and Svec,[20] in studying fermentation reactions of the saliva of 53 children, found that *L. casei* and *L. plantarum* were the only lactobacilli to ferment rhamnose. With fluorine in the Evanston water these children could have large numbers of *L. acidophilus, L. brevis, L. buchneri, L. fermenti,* and lactose-negative lactobacillus in their saliva and still be associated with a lower untreated carious surface rate. However, this is unlikely for Tilden and Svec[20] found *L. casei* alone or with other species of lactobacillus in 88 per cent of 45 children studied who had high lactobacillus counts.

It was shown in both age groups that some of the lactobacillus-negative cases had definite evidence of dental caries activity. This may be due to the fact that although there was no evidence of lactobacillus in the saliva of these students, there could have been other bacteria responsible, or lactobacillus may have been harbored in bacterial plaques on the surfaces of teeth and not dislodged when the wax pellet was chewed.

There appeared to be a variation in some of the base-line rates and the control rates. This may be due to the differences found in the make-up of the 2 groups. In a previous report from the Evanston Dental Caries Studies,[21] it was found that 22.2 per cent of the children examined in the study area were attending parochial schools while no such children were included in the control area. Also 10.0 per cent of the children in the study area were Negro children while in the control area 0.1 per cent were Negro. "Statistically significant differences were found to exist between the caries rates of Negro and Parochial school children on one hand and public white school children on the other."

There were two cases included in this report with untreated carious surfaces somewhat out of line with the others. The first case was in the control area in 1947. One of the children in the 6 to 8-year-old group in the low lactobacillus division had a total of 61 untreated carious surfaces. There was no

evidence of the child ever having had any dental treatment. The second case was in the study area in 1951. Here one of the children of the 6 to 8-year-old group in the high lactobacillus division had 67 untreated carious surfaces. There was no evidence of any restorative treatment.

SUMMARY

The results of the study of 2,235 saliva specimens contributed by specific age group children in the Evanston Dental Caries Study in relation to lactobacillus counts and untreated dental caries rates before fluoridation and for a period of 6 years and 10 months after fluoridation have been reported.

In the younger children the negative cases of the low lactobacillus division increased from 43.75 per cent before fluoridation to 57.57 per cent after 82 months of fluoridation. The younger age group with 11.90 per cent of the lactobacillus negative cases with no carious surfaces in 1946 had 26.31 per cent in 1953. The untreated carious surface rates per hundred children in the low and moderate lactobacillus divisions were considerably less in 1953 than in 1946.

In the older age group of children the negative lactobacillus cases of the low lactobacillus division increased from 39.13 per cent in 1946 to 42.63 per cent in 1952. The percentage of lactobacillus negative cases with no carious surfaces in 1946 was 5.55 per cent and 16.36 per cent in 1952. An increase of lactobacillus colony counts was noted in the older age group as in 1946, 61.60 per cent of the cases were in the low lactobacillus division while in 1952 only 46.57 per cent of the cases were found here. In 1946, 29.91 per cent of all the cases of the older children were found in the moderate lactobacillus division. In 1952, 47.65 per cent of the cases were found in this division.

The variation associated with the high lactobacillus division was attributed to the small number of cases in this division while the differences in the make-up of the control group and the study group were thought to be responsible for some of the differences in rates found in these two groups.

A relationship between the lactobacillus count and the untreated dental caries rate was noted in that the cases with low lactobacillus counts had less untreated carious surface rates than had the cases with greater lactobacillus colony counts.

It is evident from the data that shifts in the proportions of children in the high, moderate, and low lactobacillus divisions have taken place which are greater than would be expected due to chance. It is assumed that these shifts may be related to the sodium fluoride in the water supply.

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ANALYSIS AND INTERPRETATION OF DENTAL FIGURES FROM THE PENNSYLVANIA SCHOOL EXAMINATIONS, 1950-1951, 1951-1952

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GRACE[1, 2] has reported the findings from dental examinations of 1,874,067 Pennsylvania children between 6 and 18 years of age. No survey of similar scope conducted by equally reliable methods seems to be available[3, 4] and, since these data do not appear to have been subjected to detailed analysis, they are evaluated and interpreted in terms of the average child of each age.

The dental examinations were a part of State-authorized annual physical examinations of all Pennsylvania school children in alternate grades from one to eleven. Each of the two published articles[1, 2] contained a table for the year's examinations in which the combined totals for the entire state were expressed in numbers of children and numbers of teeth. Additional tables in each article provided regional summaries and indications of trends.

Each dental examination was conducted by a dentist, employing mouth mirror, chip blower and explorer under "adequate light." The rate of examination did not exceed 8 children per dental examiner per hour. Findings were announced to a recording clerk. X-ray examinations were made only exceptionally. In the 1950-51 survey, the total number of examiners throughout the State was 1200.

Findings were expressed in DMF permanent teeth. The criteria for DMF were as follows: D: A permanent tooth not requiring extraction, in which caries were present. Pre-carious pits and fissures were not counted. D included filled teeth which were carious at examination time. M: A permanent tooth which had been extracted or which required extraction. F: A permanent tooth which contained one or more fillings and no caries. A tooth with a history of caries was counted only once in the examination as D, M, or F and was therefore included only once in DMF. A tooth was considered erupted when any portion of it was through the gingiva.

The omission of routine bite-wing x-ray examinations, and the exclusion from the record of "pre-carious" lesions make it certain that a significant number of carious teeth escaped detection. The figures for D must be considered lower than the true figures, both because some carious teeth were unavoidably passed by the examiners as intact, and some filled teeth with caries were classed F instead of D. The first type of error also reduced the DMF count. In the case of F, the absence of x-ray examination resulted in a higher figure than warranted, since clinical examination would rarely fail to detect a filled tooth but would be likely to omit obscure caries in some filled teeth.

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In the figures for M, a possible source of error, in addition to absence of x-ray evidence, was lack of a stated basis for classifying a tooth as "requiring extraction." Therefore, some teeth requiring extensive restoration or root-canal therapy might have been counted as either D or M, depending on the individual examiner's opinion. However, the M figures may be considered fairly objective because (1) M figures closely corresponded in all the separate tables included in the original articles. This indicates that common standards were held by the examiners, whatever these standards were, and (2) in a survey covering great numbers of the general population, all untreated teeth that are pulp-involved or that require extensive restoration may conceivably be classed as M without risk of significant error. This assumes that the proportion of such teeth that are actually salvaged is not significant. D and F figures make it plain that less than half the filling requirements are met at any age.

The figures for the Pennsylvania Examinations may be regarded generally as conservative for D and DMF, high for F, and accurate in a practical sense for M. The errors do not invalidate the figures, since a survey of this scope and nature is intended to provide general, not detailed, information, and the earlier source-material in this field[5-12] is at least equally affected by the same types of errors. For several reasons, the figures for the Pennsylvania Examinations appear to provide the most reliable record to date of gross caries incidence and treatment in the permanent teeth of young Americans: The populations sample represented by the total figures is quite large and typical; it is unweighted in regard to sex, race, national origin, economic status, rural or urban residence, composition of water, availability of dental care, or any other factors that affect findings in smaller groups. The type of examination was the most effective one for eliciting gross clinical findings from a great number of subjects during a short period, and the conduct of examinations was fully regulated. These data are contemporary, and the two sets of annual findings provide a means of cross-checking. The Pennsylvania population may be considered at least as typically American as any used in previously published large-scale studies.

Since this paper is concerned with an appraisal of the general findings, the tables containing the state-wide data only will be considered here.

Table I presents the same data in terms of average permanent teeth per child at each age, in each category of DMF. Table I may be used as a standard by which to measure a group for caries-history, extent of current caries, tooth mortality, and level of treatment. It provides a means of putting into concrete terms, for the benefit of dentist and patient, the expected reduction in future caries-incidence following fluoridation of the communal water supply, or topical fluoride applications.

Separate consideration of each vertical column of figures in Table I will provide detailed information on DMF and its components.

DMF

The number of DMF teeth records the general caries-experience of the individual and is an accepted standard measure of the caries-experience of a

SUMMARY OF DENTAL EXAMINATIONS OF SCHOOL CHILDREN, 1950-51
(From Grace, L. G., Pa. D. J. 19: 3, 1952)[1]

AGE, NEAREST BIRTHDAY	NUMBER EXAMINED	NUMBER DECAYED PERMANENT TEETH REQUIRING FILLING	NUMBER PERMANENT TEETH EXTRACTED OR REQUIRING EXTRACTION	NUMBER PREVIOUSLY FILLED PERMANENT TEETH	TOTAL DECAYED, MISSING AND FILLED PERMANENT TEETH	AVERAGE DECAYED MISSING AND FILLED PERMANENT TEETH PER CHILD	AVERAGE FILLED PERMANENT TEETH PER CHILD
6	148,051	55,865	1,601	7,478	64,944	0.44	0.05
7	51,884	46,142	2,403	10,114	58,659	1.1	0.2
8	140,361	202,946	19,756	88,912	311,614	2.2	0.6
9	57,430	101,168	15,952	43,450	160,570	2.8	0.8
10	107,086	218,102	44,926	140,694	403,722	3.8	1.3
11	54,723	134,785	32,932	78,970	246,687	4.5	1.5
12	101,782	295,314	82,704	242,463	620,481	6.1	2.4
13	59,068	197,387	65,372	143,533	406,292	6.9	2.4
14	93,546	345,179	139,436	358,774	843,389	9.0	3.8
15	56,452	225,926	106,687	221,593	554,206	9.8	3.9
16	76,902	272,106	163,552	416,295	851,953	11.1	5.4
17	32,721	117,566	81,519	173,540	372,625	11.4	5.3
18	7,407	29,306	21,655	34,986	85,947	11.6	4.7
	987,413	2,241,792	778,495	1,960,802	4,981,089		

SUMMARY OF DENTAL EXAMINATIONS OF SCHOOL CHILDREN, 1951-52
(From Grace, L. G., Pa. D. J. 20: 3, 1953)[2]

6	129,990	47,516	1,487	8,697	57,700	0.44	0.07
7	44,230	36,864	2,090	8,502	47,456	1.1	0.19
8	119,545	167,440	18,105	79,470	265,015	2.2	0.66
9	55,545	95,428	16,703	45,403	157,534	2.8	0.82
10	103,167	196,829	49,189	144,388	390,406	3.8	1.4
11	50,312	119,449	33,470	74,829	227,748	4.5	1.5
12	88,925	250,852	73,247	229,687	553,786	6.2	2.6
13	51,917	180,910	61,042	159,234	401,186	7.7	3.1
14	85,776	307,149	122,557	343,157	772,863	9.0	4.0
15	50,301	196,645	92,612	205,290	494,547	9.8	4.1
16	71,088	244,472	145,468	397,934	787,874	11.1	5.6
17	29,889	108,049	73,772	201,516	383,337	12.8	6.7
18	5,967	22,800	18,218	30,570	71,588	12.0	5.1
	886,654	1,974,403	707,960	1,928,677	4,611,040		

TABLE I

AVERAGE PERMANENT TEETH PER CHILD
EXAMINATIONS 1950-51, 1951-52, PA.

1 AGE	2 DMF 50-51	3 DMF 51-52	4 D 50-51	5 D 51-52	6 M 50-51	7 M 51-52	8 F 50-51	9 F 51-52
6	0.44	0.44	0.371	0.351	0.011	0.011	0.05	0.07
7	1.1	1.1	0.85	0.863	0.05	0.047	0.2	0.19
8	2.2	2.2	1.46	1.39	0.14	0.15	0.6	0.66
9	2.8	2.8	1.7	1.68	0.28	0.3	0.8	0.82
10	3.8	3.8	2.1	1.94	0.42	0.46	1.3	1.4
11	4.5	4.5	2.4	2.34	0.6	0.66	1.5	1.5
12	6.1	6.2	2.9	2.8	0.8	0.8	2.4	2.6
13	6.9	7.7	3.4	3.43	1.2	1.17	2.4	3.1
14	9.0	9.0	3.7	3.6	1.5	1.4	3.8	4.0
15	9.8	9.8	3.9	3.9	2.0	1.8	3.9	4.1
16	11.1	11.1	3.6	3.5	2.2	2.0	5.4	5.6
17	11.4	12.8	3.6	3.7	2.5	2.4	5.3	6.7
18	11.6	12.0	3.9	3.9	3.0	3.0	4.7	5.1

group.[13, 14] The two annual reports are identical to age 12. The higher 1951-52 figures at ages 13, 17, and 18 are not considered significant for the present study.

The DMF reveals a situation which in general is as follows: In the average Pennsylvania child, one-half of one permanent tooth is affected by caries at age 6. In the next 12 years, 11 more teeth are involved, an average of nearly 1 additional permanent tooth per year. At age 18, two-fifths of the permanent dentition of the average child has been affected by caries.

The extent of the carious attack in the early years may not be seen at first glance in these figures. In evaluating DMF at ages under 12, the number of permanent teeth erupted must be taken into account. $\frac{DMF}{n}$, where n equals the number of teeth erupted,[15] should be the basis of comparison among early DMF figures, and for any comparison of these with DMF at ages 13 and above. Since this information was not included in the present examinations, this factor can only be estimated from accepted average eruption-ages.[16] An example may serve to make plain the way in which DMF in the early ages should be appraised:

At age 8, normally 12 permanent teeth may be considered "erupted" according to the definition given, while at age 14, there are normally twenty-eight. Comparison is made between $\frac{2.2\ DMF}{12\ n}$ at age 8, and $\frac{9\ DMF}{28\ n}$ at age 14. On this basis, the DMF at age 8 is not one-quarter of the DMF at age 14, as would appear at first; it is over one-half.

DM

D and M express the need for dental care. D, caries, represents operative need and M, tooth mortality, represents surgical and prosthetic need. As with DMF, figures for D and M in 1950-51 and 1951-52, generally coincide at each age, with some exceptions noted.

D shows an unbroken rise from ages 6 to 15. This indicates that operative need continually out-distances supply (F), even though F likewise rises. D must be considered a measure of the inadequacy of F, even though D includes some teeth with 1 or more filled surfaces.

Table I shows that the average 6-year-old has untreated caries in one-third of one permanent tooth. At age 8, this figure is 1½ teeth; at age 10, 2; at age 12, 3; at age 14, 3½; and at age 15, almost 4. At ages 16 and 17, D is slightly below age 15, despite the rising DMF, and D at age 18 equals age 15.

Table II shows the changes in D, M and F at each age in the average child. It aids a closer study of the interdependence of the 3 components of the caries-history.

The drop in D at age 16 may be compared in Table II to the rise in F at this age—the highest rise in the record. At age 17, in the 1951-52 figures, the DMF and F are atypically high, and D is still below age 15. However, the increase of M by one tooth, from ages 15 to 18, may be considered the major factor in the leveling-off of D in this age-group.

In the young, the greatest percentage of permanent tooth loss is due to caries, particularly neglected caries. At ages 6 to 18, M may be considered

TABLE II

YEARLY CHANGES IN D, M AND F
(AVERAGES OF 1950-51 AND 1951-52 EXAMINATIONS)
AVERAGE PERMANENT TEETH PER CHILD

AGE	D	M	F
7	plus 0.495	plus 0.0375	plus 0.13
8	plus 0.56	plus 0.09	plus 0.435
9	plus 0.27	plus 0.145	plus 0.18
10	plus 0.28	plus 0.15	plus 0.54
11	plus 0.40	plus 0.19	plus 0.15
12	plus 0.48	plus 0.17	plus 1.0
13	plus 0.56	plus 0.38	plus 0.2
14	plus 0.24	plus 0.27	plus 1.2
15	plus 0.25	plus 0.45	plus 0.1
16	minus 0.35	plus 0.2	plus 1.5
17	plus 0.10	plus 0.35	plus 0.5
18	plus 0.25	plus 0.55	minus 1.1

almost entirely a product of D.[17, 18] Statistically, M does not parallel DMF or D, but it follows in the wake of D. At age 6, the average child has a tooth mortality of one one-hundredth of one permanent tooth. This is equal to 3 per cent of his dental requirements (D plus M) at this age. At age 18, the mortality is 3 teeth, and equals 45 per cent of his dental requirements.

In Table II, it may be seen that M approximately triples itself to age 7, and again to age 8, equalling at age 8, one-tenth of one permanent tooth. To age 11, the rise in M each year, in terms of number of teeth, is half as high as the rise in D. By age 13, it equals 1 tooth. At each age from 14 to 18, M rises more steeply than D. Two permanent teeth are lost by age 15, and 3 teeth by age 18.

M accumulates to age 12 and manifests after that age. Tooth mortality will logically claim the teeth first attacked by caries, since these teeth may also be the longest neglected or the most frequently re-attacked. Findings of several surveys of tooth mortality[19-21] are unanimous on the high proportion of first permanent molars within the total numbers of missing teeth in young Americans. Likewise, numerous investigators are agreed that the first permanent molars, of all permanent teeth, are the most highly susceptible to caries.[22-24]

F

F totals in the Pennsylvania Examinations include the number of filled teeth which do not have clinical caries at examination time. No separate records were made of filled teeth with new caries, or of number of fillings, which would give an indication of filling-history, or what might be termed treatment-effort. As with the previous categories, F records in 1950-51 and 1951-52 generally coincide at each age.

At all ages, F is far from adequate, never constituting half the DMF. The total of D and M at each age is, in fact, the accurate measure of the inadequacy. During the early ages, when filling operations may constitute the most effective long-term preventive treatment, F is only a small percentage of the rapidly rising DMF. F is too little and too late. It is too little at ages up to 9, and thereafter it is too late to be preventive in the fullest sense.

In the permanent teeth of the average child at age 6, F is equivalent to only one-sixth of the operative need. In spite of the accumulation of M, D totals show a steeper rise than F totals to age 9. At age 10, the F rise exceeds D rise, and the average child has approximately 1½ teeth filled. At age 12, the total F has risen to 2½ teeth, almost one-half the operative need at this age. After this age, D remains at a figure below 4 teeth, while F shows a yearly increase, more marked in the 1951-52 examinations, to age 17, when it is an average of 6 teeth. The drop to 5 teeth at age 18 is attributable to the increase in M, at this age, to 3 teeth. At age 18, the average caries-history or DMF involves 12 teeth. Three are lost (M), approximately 5 are filled (F) and 4 are in need of filling (D).

Although new caries in filled teeth statistically conceal fillings, actual distortion in a survey of these dimensions is not possible, and the low F figures may not be construed as a statistical illusion. Such a possibility exists only among small numbers of individuals with rampant caries, examined over a period of a few years, where annual recurrent caries in many filled teeth conceals F appreciably. The low F at age 6, in these studies, the rise in F each year to age 17, and the comparison between D and F rises each year, preclude this sort of wholesale distortion. F figures plainly reveal a low ratio of filled teeth to caries incidence.

To increase the ratio of F, efforts must be expended in 3 directions: (1) greater numbers of teeth must be filled at all ages from 6 to 18. At age 6, the requirement is for 6 times the number of existing fillings. (2) The fillings placed in young permanent teeth must include susceptible areas as well as those already involved by caries. This is the principle of extension for prevention. Technically superior operative work is required for children at early ages.[3] Proved methods for preventing, retarding, and inhibiting the incidence of new caries must be practiced on a vastly greater scale.

The conclusions of Finn are verified by these findings: "There is an appalling disparity between dental needs and dental corrections today. . . . This difference becomes greater with age. The high dental mortality rates (among school children) are a result of this neglect. Not only is the accumulated problem not being met, but the yearly increment of new cavities is not being adequately treated. The time required to rehabilitate the mouth increases with age and with the ability to secure adequate dental attention."[25]

SUMMARY AND CONCLUSIONS

The dental examinations in the School Health Examinations in Pennsylvania and their presentation are evaluated as an excellent source of current authentic data on the general caries-prevalence, level of treatment and tooth mortality in permanent teeth in the 6- to 18-year-old school population of the state. An analysis in terms of numbers of teeth in the average child at each age reveals:

(1) High total caries-experience, marked by early onset, with early multiple-tooth involvement.

(2) Low ratio of filled teeth to operative need at every age.

(3) High tooth mortality.

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THE DRYOPITHECUS PATTERN IN RECENT DANES AND DUTCHMEN

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THE CONCEPT OF THE "DRYOPITHECUS PATTERN"

GREGORY[3] gave a detailed description of the pattern of the lower molars of Dryopithecus. His characterization contained the following details (pp. 293-294):

> There are five main cusps, three of which (protoconid, hypoconid, mesoconid) are on the external side of the crown and two (metaconid, entoconid) on the internal side. The metaconid, which is the highest cusp, is directly internal to the protoconid; the hypoconid is opposite the valley between the metaconid and the entoconid; the mesoconid (or hypoconulid) is on or near the postero-median border of the tooth, behind the hypoconid and entoconid. The external basal cingulum is more or less reduced but sometimes persists opposite the posterior part of the protoconid. In front of the protoconid and metaconid is a remnant of an earlier trigonid basin in the form of a transverse valley; into this depression fitted the hypocone of an upper molar. At the back of the tooth there is a very thick cingulum which sometimes is confluent with the mesoconid. The surface of the lower molar crown is likewise characterized by the arrangement of certain furrows; the hypoconid is limited anteriorly and posteriorly by two deep transverse furrows which converge into a prominent inverted V (∧), the narrow end of which is at the centre of the crown. From the narrow end and sides of this truncated V, other furrows radiate as follows: (a) an anterior central furrow between the protoconid and metaconid, (b) a posterior central furrow between the mesoconid and entoconid, and (c) one or two internal furrows between the metaconid and entoconid.

Further, Gregory demonstrated that this fundamental pattern is characteristic of the lower molars in Simiidae and Hominidae, but that it is more or less obscured in the different forms by secondary modifications.

In comparative studies within the order of Primates, it is of importance to remember that it is the whole of this pattern, not merely parts thereof, which is characteristic of defined racial groups. This is emphasized here because in the last decades there has been a pronounced tendency to regard the Dryopithecus pattern merely as a certain combination of cusps and grooves, and a tendency to forget that this combination alone (or its special modifications) does not characterize hominids and simiids.

Gregory's definition of the Dryopithecus pattern gave rise to important comparative dental anatomic studies.[4-8]

In phylogenetic studies it must always be remembered that the various structural "elements" of any one organ may be modified in a different degree, as some elements are more conservative than others. It is very often more

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difficult for the phylogenetists to define which structural element is to serve as a basis for the classification than to determine what is primitive and what is modified. Dental morphologists may base their classification on different points of view. Some will use the mesiodistal length of the dental arch, the frequency of agenesis of third molars, or the number of cusps; others, again, the developmental degree of Carabelli's cusp, of the external basal cingulum on the lower molars, and so forth, and will assert that the more modified the morphologic characteristic is, the more progressive is the group of individuals concerned. Such an argument will very easily lead to erroneous conclusions, since those classifications which can be made on the basis of the degree of modification of individual structures are not always identical. Consequently, morphologic analysis of individual anatomic structures must not necessarily lead to an immediate phylogenetic synthesis. A great number of structures must be analyzed separately, compared, and weighed against each other before a probable synthesis can be formed.

When studying the modification degree of the Dryopithecus pattern in recent man, one must, like Gregory, appreciate the fact that this pattern is composed of individual structures, each of which leads a more or less independent existence, even within the same dentition.

If the groove pattern and the cusp number are regarded as two elementary structures in the Dryopithecus pattern of the lower molars, a priori, we must analyze these elements separately. Hellman[8] examined the relative frequency and modification of these two elements on the three lower molars in a considerable number of ethnic groups. He divided the changes in the Dryopithecus pattern into four successive stages: *Stage 1,* representing the original Dryopithecus pattern indicated by the sign Y5. *Stage 2,* in which the primitive cusp number is reduced from 5 to 4; the Y-shaped groove pattern is still retained, but with the loss of the oblique line in the pattern. As in Stage 1, there is contact between cusps No. 2 and 3. The pattern is indicated by (half) Y4. *Stage 3,* in which the number of cusps (5) is primitive, but the groove pattern changed from Y shape to + shape; the basal contact of Cusps 2 and 3 is lost. The pattern is indicated by +5. *Stage 4,* in which both the number of cusps and the groove pattern are modified. The aggregate pattern is indicated by +4. More recent authors[1, 2, 9-11] have followed Hellman's classification of the lower molar patterns.

Unfortunately, Hellman failed to explain why he considered the pattern Y4 to be more primitive than +5, nor have subsequent writers accounted for sharing this view. Dahlberg[1] attempted a classification with phylogenetic equivalence for the two transitional patterns. Steslicka[11] lumped them in one group, which she termed "median." The difficulties of classifying the transitional forms from the primitive Y5 pattern to the most modified pattern indicated by the symbol +4 are circumvented by observing groove pattern and cusp number separately.

On examination of the correlation between groove pattern and cusp number, it is at once apparent that this is very low in almost every case. In Fig. 2

the correlation ratio between the two morphologic elements is represented graphically. Each point represents a population's mean lower molar (first, second, or third). The percentage frequency of the primitive Y-shaped groove pattern for each lower molar in a racial group is represented by the ordinate, while the percentage frequency of the primitive cusp No. 5 is represented by the abscissa. The point at the top to the right in the coordinate system represents the highest degree of primitiveness with 100 per cent Y pattern and 100 per cent five-cusped molars, while the point at the bottom to the left with 0 per

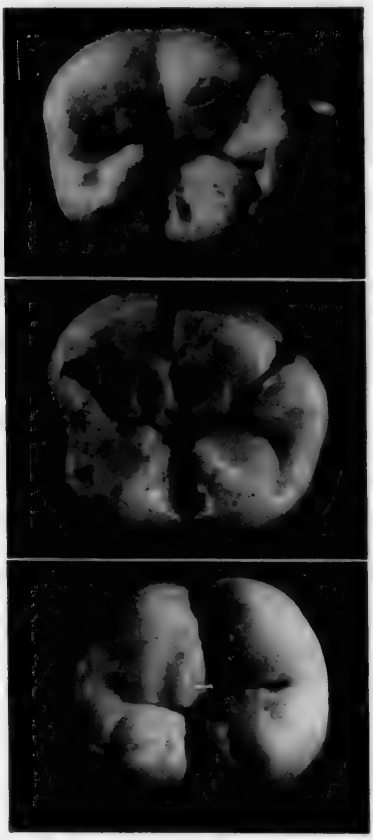

Fig. 1.—The three principal types of the occlusal groove pattern on the lower molars. *A*, Y pattern (M_1 inf. dex.), *B*, + pattern (M_1 inf. dex.), *C*, X pattern (M_2 inf. dex.).

cent Y (that is, 100 per cent + pattern) and 0 per cent five-cusped forms (that is, 100 per cent four-cusped forms) corresponds to the highest degree of modification in regard to these distinctive characters. From the graphic representation it is apparent that for the majority of the lower molars in the groups, the Y pattern is more recessive than the primitive cusp number or, expressed in a different way, the primitive cusp number is generally a more conservative

morphologic feature than the primitive Y pattern. This is reason enough for treating the two structural elements separately instead of collectively, as has been done hitherto.

The characteristic of the change from Y pattern to + pattern is a relative distal shifting of the groove between the protoconid and hypoconid. In this distal shifting may be distinguished three well-defined stages (Fig. 1, *A*, *B*, and *C*): *Stage 1* is the primitive Dryopithecus stage with linear, basal contact between hypoconid-metaconid, symbolized by the sign Y. *Stage 2* is characterized by a basal, point-shaped contact between hypoconid-metaconid and protoconid-entoconid. The term "point-shaped contact," as used here, means that it cannot be determined with certainty which of the two diagonal pairs of cusps has most basal contact, or that this contact does not exceed 0.2 mm. This stage is symbolized by the sign "+." *Stage 3* is the most modified, or most advanced, stage. Here there is a basal, linear contact between the cusps protoconid-entoconid. It is symbolized by the sign "X."

As will be seen, the sign "+" in Hellman's system corresponds to the stages "+" and "X" referred to here. The present classification is used because of the desirability of classifying in as many details as possible the evolutionary progress on the basis of the morphology of the teeth, and to the fact that all three well-defined forms of the groove pattern were observed with reasonable frequency.

THE MATERIAL

My own observations have been made on teeth of recent Danes and Dutchmen. The Danish material originates from two medieval burial places, the site of Æbelholt monastery near the town of Hillerød and a cemetery for lepers at Åderup near the town of Næstved. All this material was excavated with great care and skill by Dr. V. Møller-Christensen.* Both localities are situated in the island of Zealand, and no reason is known to explain why the individuals which were examined should be morphologically different from the population now living in the island. The specimens from the two Danish localities are treated together. The Dutch material comprises the well-known, large tooth collection of modern Amsterdamers in the Anatomo-Embryological Institute in Amsterdam. This material was recovered at the initiative of the late Professor L. Bolk, who, in connection with the closing of two large cemeteries in Amsterdam, organized a large-scale collection of anatomically noteworthy skeletal remains. From a very great number of skulls with complete dentitions, but otherwise defective, the teeth were extracted and sorted out according to type, so that all right and left mandibular first molars, for example, are kept in one container. As those discontinued burial grounds were not special men's or women's cemeteries, it must be presumed that this Dutch material of extracted teeth is distributed between males and females.

The extent of the material examined is shown in Table I.

*I am much indebted to Dr. Møller-Christensen, Roskilde, Dr. Th. E. de Jonge, and Professor Dr. M. W. Woerdemann, both of Amsterdam, for assistance and permission to carry out the observations.

This summary includes all the teeth on which at least one of the structural elements, groove pattern or cusp number, in the original or modified Dryopithecus pattern could be determined. In accordance with my views, the different structural elements have been dealt with separately, and therefore it has not been necessary to exclude teeth where only one of those elements could be identified.

TABLE I

SURVEY OF THE MATERIAL OF LOWER MOLARS EXAMINED IN RECENT DANES AND DUTCHMEN

	INDIVIDUALS	FEMALES	MALES	UNDETERMINED SEX	M_2 SIN.	M_2 SIN.	M_1 SIN.	M_1 DEX.	M_2 DEX.	M_3 DEX.
Danish	336	52	53	231	137	212	150	150	217	145
Dutch	---	--	--	---	488	712	792	832	734	497

THE DISTRIBUTION OF THE PATTERN TYPES IN THE MATERIAL

Table II shows the frequency of the groove patterns Y, +, and X, of the cusp number, and of the patterns Y5, Y4, +5, and +4 in recent Dutchmen.

TABLE II

THE FREQUENCY OF THE DIFFERENT OCCLUSAL PATTERNS ON THE LOWER MOLARS IN MODERN DUTCHMEN (♂ + ♀)

		M_3 SIN.	M_2 SIN.	M_1 SIN.	M_1 DEX.	M_2 DEX.	M_3 DEX.
Y	Number	50	138	480	466	113	51
	Per cent	11.2	21.0	76.5	69.2	16.7	10.9
+	Number	44	166	92	122	181	57
	Per cent	9.9	25.2	14.7	18.1	26.8	12.2
X	Number	352	354	55	86	382	359
	Per cent	78.9	53.8	8.8	12.7	56.5	76.9
5 cusps	Number	291	97	679	721	82	258
	Per cent	60.6	14.0	89.8	89.4	11.6	52.3
4 cusps	Number	189	594	77	86	625	235
	Per cent	39.4	86.0	10.2	10.6	88.4	47.7
Y_5	Number	22	22	401	396	12	17
	Per cent	5.0	3.4	67.4	61.2	1.8	3.7
Y_4	Number	28	112	51	50	99	34
	Per cent	6.4	17.6	8.6	7.7	15.2	7.3
$+_5$	Number	242	66	119	177	66	223
	Per cent	55.0	10.3	20.0	27.4	10.1	48.1
$+_4$	Number	148	438	24	24	474	190
	Per cent	33.6	68.7	4.0	3.7	72.8	40.9

The primitive Y pattern is found much more frequently on the first molar (M_1) than on the second (M_2), and on the latter, again, more frequently than it is on the third molar (M_3). There is a very clear difference in the degree of modification of the Y pattern: M_1 is most primitive, M_3 most modified, while M_2 occupies an intermediary stage more nearly approaching M_3. The reverse is true of the X pattern, which occurs most frequently on M_3, while it is just as rare on M_1 as the Y pattern is on M_3. The difference in the Y pattern between the three molars is very clear, and the difference between M_1 and M_2 is distinctly greater than that between M_2 and M_3. The + pattern may be regarded on the one hand as the highest evolutionary degree of the Y pattern and on the other as an incipient X pattern. So in teeth such as M_2, where the modification

degree of the primitive Y pattern is still in an intermediate stage, it is not surprising to find a relatively high frequency of the + pattern, as expressed in Table II.

The system adopted here for classification of the groove patterns in the lower molars is only to be regarded as a means for a rough estimation of the modification degree of the original pattern. In the primitive Dryopithecus groove pattern, the basal contact of hypoconid-metaconid (''the 2-3 contact'') is very broad. This is, for example, plainly seen on M_1 and M_2 in the specimen type of *Dryopithecus frickae,* shown in Hellman's[8] Fig. 2. In the material of recent Danes and Dutchmen, a similar broad 2-3 contact is of rather rare occurrence in the Y pattern on M_1; and on M_2 and M_3 with Y pattern, the contact is in most cases essentially shorter than it is on M_1. The material presented statistically in Table II as Y pattern does not signify the same for M_1, M_2, and M_3. The Y pattern on M_2 or M_3 is more modified, that is, approaches the + pattern more than it does on M_1, and the two posterior molars are even more progressive than can be revealed by the table. Similar conditions apply to the X pattern: a broad basal contact between the protoconid and entoconid (''the 1-4 contact'') is rare on M_1 with X pattern, comparatively frequent on M_2 with X pattern, but the general rule on M_3.

According to Table II, the primitive cusp number, 5, is present in about 90 per cent of M_1, but only in about 13 per cent of M_2. On M_3, more than one-half the teeth have retained five cusps. There seems to be a discrepancy between the modification degree of the three molars, and the distribution of the groove patterns. A discussion is hardly warranted as long as the genetic factors which determine the number of cusps are not more clarified. The hypoconid on M_2 may be reduced in size, so that it is smaller than the hypoconulid. In the Amsterdam material it was possible to draw up a morphologic sequence which showed the gradual reduction and disappearance of the hypoconid, the final result being a four-cusped m_2, whose distofacial cusp was homologous with the hypoconulid. Similar conditions could be observed in M_3. It is not possible, of course, on the basis of such an observation to determine whether a four-cusped tooth may result from a reduction of the hypoconid, or whether it is a question of the formation of a five-cusped tooth by progressive development of a new cusp, which is not homologous with any cusp in the primitive Dryopithecus tooth form. Attention must also be drawn to the third molar's special location and function, which, perhaps, should induce a more conservative attitude toward the reduction of the hypoconulid. At any rate, a phylogenetic interpretation of the cusp number in this region of the dentition must be given with caution.

If the teeth in the left and right sides are compared, it is at once evident (Table II) that the Y pattern occurs much more frequently on the first molar in the left side (M_1 sin.) than on the first molar in the right side (M_1 dex.). The difference between the two frequencies (76.5 − 69.2 = 7.3) is almost significant, as $3 \cdot \epsilon_{diff.} = 7.37$. This means that the probability that the difference found in the frequency of the Y pattern on the right and left side first molars should be accidental is less than 0.3 per cent.

Between the right and left M_2, there is a difference in the frequency of the Y pattern of a similar quality as that between the left and right M_1. The difference here is, however, less pronounced. In M_3, on the other hand, no difference has been shown between right and left sides. The frequency of five cusps is about the same in the right and left sides in M_1 and M_2, but there is a pretty certain difference between the right and left M_3: greatest frequency (60.6 ± 2.2 per cent) in the left side, at least (52.3 ± 2.2 per cent) in the right.

The knowledge of these divergencies between the right and the left side may be of little anthropologic value in itself, but it shows that in comparative studies between different populations, or between species and genera, it is necessary to make clear from which side the molars in question originate.

Table II includes the statistical frequency of Hellman's four patterns, Y5, Y4, +5, and +4, on the Dutch lower molars. This is merely for the purpose of continuity with the observations of previous authors. I will refrain from any comments on these figures.

In Table III the statistical data relating to the Danish material are tabulated. The comments in this table will be much the same as those on Table II. It must be emphasized, however, that no statistically valid differences could be shown between the teeth in the right and left sides in the Danish series. It has been possible to determine the sex of only a small number of the individuals examined. This is due partly to their infancy, partly to underdevelopment of the secondary sexual characters or defects in the adult skeletal

TABLE III

THE FREQUENCY OF THE DIFFERENT OCCLUSAL PATTERNS ON THE LOWER MOLARS IN MEDIEVAL DANES

		M_2 SIN.	M_2 SIN.	M_1 SIN.	M_1 DEX.	M_2 DEX.	M_2 DEX.
Y	Number	10	44	101	102	31	16
	Per cent	7.4	20.9	69.2	69.4	14.3	11.2
+	Number	21	52	20	18	59	25
	Per cent	15.6	24.6	13.7	12.2	27.2	17.5
X	Number	104	115	25	27	127	102
	Per cent	77.0	54.5	17.1	18.4	58.5	71.3
5 cusps	Number	75	17	140	135	17	79
	Per cent	58.1	10.5	97.9	95.1	10.4	59.4
4 cusps	Number	54	145	3	7	146	54
	Per cent	41.9	89.5	2.1	4.9	89.6	40.6
Y_5	Number	4	3	89	82	1	8
	Per cent	3.1	1.9	65.0	60.7	0.6	6.2
Y_4	Number	15	30	3	5	22	8
	Per cent	11.5	18.5	2.2	3.7	13.5	6.2
$+_5$	Number	67	14	45	46	16	70
	Per cent	51.1	8.6	32.8	34.1	9.8	54.3
$+_4$	Number	45	115	0	2	124	43
	Per cent	34.4	71.0	0.0	1.5	76.1	33.3

material. Only fifty-three male and fifty-two female individuals could be sexed. For these two subgroups it was not possible to show clear differences between homologous teeth. The small numerical data on which this information is based are not presented here.

If the Danish and Dutch series (Tables II and III) are compared, it will be seen that there is an essential difference between the two groups in the frequency of five-cusped first molars. The statistical calculation shows that

TABLE IV

THE RESULTS OF THE PREVIOUS KNOWN OBSERVATIONS ON THE MODIFICATION DEGREE OF THE DRYOPITHECUS PATTERN OF THE FIRST MOLAR IN MAN

POPULATION	NO.	NUMBER		Y_5 (%)	Y_4 (%)	$+_5$ (%)	$+_4$ (%)	Y (%)	+ (%)	5 CUSPS (%)	4 CUSPS (%)	NOTES
		INDIVIDUALS	TEETH									
Australians	1	20	18	100.0	0.0	0.0	0.0	100.0	0.0	100.0	0.0	Hellman, 1928
Buriat Mongols	2	21	36	100.0	0.0	0.0	0.0	100.0	0.0	100.0	0.0	Hellman, 1928
Eskimos	3	30	29	97.0	0.0	3.0	0.0	97.0	3.0	100.0	0.0	Hellman, 1928
Alaska Eskimos	4		67	89.6	1.5	6.0	3.0	91.1	9.0	95.6	4.5	Goldstein, 1948
East Greenland Eskimos	5		169	95.8	0.0	3.0	1.2	95.8	4.2	98.8	1.2	Pedersen, 1949, models and mandibles
East Greenland Eskimos	6		141	95.7	0.0	2.8	1.4	100.0	0.0	100.0	0.0	Pedersen, 1949, models alone
Chinese	7	19	26	100.0	0.0	0.0	0.0					Hellman, 1928
Amerinds	8	55	97	100.0	0.0	0.0	0.0	100.0	0.0	100.0	0.0	Hellman, 1928
Pima Indians	9		162	99.4	0.0	0.6	0.0	99.4	0.6	100.0	0.0	Dahlberg, 1949
Pecos Indians	10		332	88.6	0.0	10.8	0.6	88.6	11.4	99.4	0.6	Nelson, 1938
Texas Indians	11		160	68.7	0.6	30.6	0.0	69.3	30.6	99.3	0.6	Goldstein, 1948
West African Negroes	12	49	97	99.0	1.0	0.0	0.0	100.0	0.0	99.0	1.0	Hellman, 1928
Uganda Negroes	13	100	161	89.0	9		2.0					Steslicka, 1948
American Negroes	14	119	179	98.0	1.0	1.0	0.0	99.0	1.0	99.0	1.0	Hellman, 1928
Jews, Lublin	15	32	36	78.0	0.0	0.0	22.0	78.0	22.0	78.0	22.0	Steslicka, 1948
Hungarians	16	42	54	83.0	11.0	0.0	6.0	94.0	6.0	83 0	17.0	Hellman, 1928
Poles	17	35	42	43.0	26		31.0					Steslicka, 1948
American children	18	85	164	74.0	12.0	13.0	1.0	86.0	14.0	87.0	13.0	Hellman, 1928
Chicago whites	19		75	84.0	8.0	2.0	2.0	92.0	4.0	86.0	10.0	Dahlberg, 1949, sum not 100 per cent
European whites	20	61	98	87.0	7.0	2.0	4.0	94.0	6.0	89.0	11.0	Hellman, 1928
Modern Dutchmen	21		832					69.2	30.8	89.4	10.6	Dreyer Jørgensen, right side
Modern Dutchmen	22		792					76.5	23.5	89.8	10.2	Dreyer Jørgensen, left side
Medieval Danes	23		150					69.4	30.8	95.1	4.9	Dreyer Jørgensen, right side
Medieval Danes	24		150					69.2	30.6	97.9	2.1	Dreyer Jørgensen, left side

Hellman stated that all the individuals examined by him were males; from the proportion between the number of individuals and teeth, it can be inferred that he included both right and left side teeth in his statistical data.

Goldstein did not distinguish between males and females in his statistical data, nor did he tell whether he had included both right and left side teeth.

Pedersen, Nelson, Dahlberg, and Steslicka did not distinguish between males and females in their statistical data, nor between right and left sides.

Dahlberg stated "number of individuals," but under this heading he entered other writers' "number of teeth."

It is not quite clear from Steslicka's English text whether the Poles are Slavs or Jews.

this difference is real. Counting of cusps is a task whose objectivity is not sufficiently reliable, and it is therefore necessary to comment upon the result of the statistical inference. The Danish first molars chiefly are unworn or slightly worn teeth, while the Dutch specimens, on the whole, are more worn. As the hypoconid-hypoconulid groove on certain forms of first molars becomes blurred and disappears with a slight degree of wear, it is probable that the differences between the Danish and Dutch first molars may be ascribed to some extent to the different degree of wear.

This deficiency applies not only to the numerical data in connection with the observation referred to here but to all specimens on which observations on

TABLE V

THE RESULTS OF THE PREVIOUS KNOWN OBSERVATIONS ON THE MODIFICATION DEGREE OF THE DRYOPITHECUS PATTERN OF THE SECOND MOLAR IN MAN

POPULATION	NO.	NUMBER INDIVIDUALS	NUMBER TEETH	Y_5 (%)	Y_4 (%)	$+_5$ (%)	$+_4$ (%)	Y (%)	+ (%)	5 CUSPS (%)	4 CUSPS (%)
Australians	1	20	21	5.0	0.0	43.0	52.0	5.0	95.0	48.0	52.0
Buriat Mongols	2	21	39	0.0	5.0	31.0	64.0	5.0	95.0	31.0	69.0
Eskimos	3	30	30	6.0	7.0	37.0	50.0	13.0	87.0	43.0	57.0
Alaska Eskimos	4		132	12.8	3.0	63.8	20.5	15.8	84.3	76.6	23.5
East Greenland Eskimos	5		128	18.7	4.7	42.2	34.4	23.5	76.6	60.9	39.1
East Greenland Eskimos	6		100	19.0	4.0	42.0	35.0				
Chinese	7	19	21	0.0	0.0	19.0	81.0	0.0	100.0	19.0	81.0
Amerinds	8	55	99	1.0	0.0	31.0	69.0	1.0	100.0	32.0	69.0
Pima Indians	9		89	2.0	1.0	69.0	28.0	3.0	97.0	71.0	29.0
Pecos Indians	10		313	8.3	1.3	24.3	66.1	9.6	90.4	32.6	67.4
Texas Indians	11		206	1.5	3.4	26.2	68.9	4.9	95.1	27.7	72.3
West African Negroes	12	49	96	17.0	12.0	8.0	63.0	29.0	71.0	25.0	75.0
Uganda Negroes	13	100	157	22.0		12	66.0				
American Negroes	14	119	218	6.0	21.0	24.0	49.0	27.0	73.0	30.0	70.0
Jews, Lublin	15	32	49	6.0	0.0	0.0	94.0	6.0	94.0	6.0	94.0
Hungarians	16	42	54	2.0	9.0	11.0	77.0	11.0	88.0	13.0	86.0
Poles	17	35	46	4.0	0.0	0.0	96.0	4.0	96.0	4.0	96.0
American children	18	85	95	0.0	4.0	6.0	90.0	4.0	96.0	6.0	94.0
Chicago whites	19										
European whites	20	61	110	0.0	5.0	1.0	94.0	5.0	95.0	1.0	99.0
Modern Dutchmen	21		734					16.7	83.3	11.6	88.4
Modern Dutchmen	22		712					21.0	79.0	14.0	86.0
Medieval Danes	23		217					14.3	85.7	10.4	89.6
Medieval Danes	24		212					20.9	79.1	10.5	89.5

Notes and comments as in Table IV.

the Dryopithecus pattern have been made. This alone makes a further analysis of the results of such cusp counts extremely difficult. In addition, the various writers' conception of the term "cusp" varies rather considerably.[10]

Objections of a similar nature may hardly be raised in respect to observations of the occlusal groove pattern. Wear on the occlusal surfaces may cause this pattern to become blurred, but may scarcely lead to systematic misinter-

pretations. If the three pattern types (Y, +, and X) are defined as mentioned previously, in every few cases will there be doubt concerning to which type a certain molar should be assigned. Therefore a classification of observed ethnic groups can, under otherwise identical mathematical conditions, be made with greater confidence on the basis of the groove pattern than on the basis of the cusp number.

TABLE VI

THE RESULTS OF THE PREVIOUS KNOWN OBSERVATIONS ON THE MODIFICATION DEGREE OF THE DRYOPITHECUS PATTERN OF THE THIRD MOLAR IN MAN

POPULATION	NO.	NUMBER		Y_5 (%)	Y_4 (%)	$+_5$ (%)	$+_4$ (%)	Y (%)	+ (%)	5 CUSPS (%)	4 CUSPS (%)
		INDIVID-UALS	TEETH								
Australians	1	20	23	14.0	0.0	72.0	14.0	14.0	86.0	86.0	14.0
Buriat Mongols	2	21	31	0.0	0.0	77.0	23.0	0.0	100.0	77.0	23.0
Eskimos	3	30	29	0.0	0.0	52.0	48.0	0.0	100.0	52.0	48.0
Alaska Eskimos	4		59	20.4	0.0	69.5	10.2	20.4	79.7	89.9	10.2
East Greenland Eskimos	5		63	15.9	7.9	73.0	3.2	23.8	76.2	88.9	11.1
East Greenland Eskimos	6		46	15.2	10.9	73.9	0.0				
Chinese	7	19	16	0.0	0.0	50.0	50.0	0.0	100.0	50.0	50.0
Amerinds	8	55	84	0.0	5.0	75.0	19.0	5.0	94.0	75.0	24.0
Pima Indians	9		7	0.0	0.0	57.0	14.0	*	*	*	*
Pecos Indians	10		249	8.4	4.8	51.0	35.8	13.2	86.8	59.4	40.6
Texas Indians	11		91	12.1	6.6	47.3	34.1	18.7	81.4	59.4	40.7
West African Negroes	12	49	88	20.0	3.0	59.0	17.0	23.0	76.0	79.0	20.0
Uganda Negroes	13	100	137	20.0		39		41.0			
American Negroes	14	119	187	4.0	3.0	63.0	30.0	7.0	93.0	67.0	33.0
Jews, Lublin	15	32	31	26.0		13		61.0			
Hungarians	16	42	35	6.0	11.0	34.0	49.0	17.0	83.0	40.0	60.0
Poles	17	35	34	5.0		21		74.0			
American children	18										
Chicago whites	19										
European whites	20	61	74	4.0	0.0	34.0	62.0	4.0	96.0	38.0	62.0
Modern Dutchmen	21		497					10.9	89.1	52.3	47.7
Modern Dutchmen	22		488					11.2	88.8	60.6	39.4
Medieval Danes	23		145					11.2	88.8	59.4	40.6
Medieval Danes	24		137					7.4	92.6	58.1	41.9

*Irregular 28 per cent. Otherwise notes and comments as in Table IV.

COMPARISON WITH PREVIOUS OBSERVATIONS

Tables IV, V, and VI present the results of previous known observations on the Dryopithecus pattern. Until now, differences between males and females or between right and left sides have not been determined systematically. The literature contains little information on the quality of the material on which the studies are based, nor does it supply precise definitions of the concepts. When all previous writers apparently base their statistical data on the pooled material of left and right side teeth, the result is a statistically incalculable change of the standard deviation, which makes a new comparison of the figures very difficult.

Thus Hellman,[8] investigating thirty-six first molars of Buriat Mongols, found 100 per cent with Y pattern and 100 per cent with five cusps. If the figure, 36, is used to express the number of individuals examined, the probability of finding within this population an arbitrary group with less than 84.9 per cent Y-patterned and 84.9 per cent five-cusped first molars is less than 0.3 per cent (the significance limit being 3 δ). But if the figure, 18, is used as a more reliable expression for the number of individuals examined (that is, the material consists of 18 right and 18 left side first molars), the significance limit will fall from 84.9 per cent to 72.0 per cent.*

In order to convey a more immediate impression of the variation of one of the morphologic elements characterizing the modified Dryopithecus pattern, I have added Table VII, which shows the frequency of the groove patterns Y, +, and X on the first molar in the right side (Table VIIa) and the left side (Table VIIb) in recent Dutchmen in groups of 100, selected at random.

TABLE VII

THE FREQUENCY OF THE GROOVE PATTERNS Y, +, AND X ON THE FIRST LOWER MOLAR IN RECENT DUTCHMEN IN GROUPS OF 100

Table VIIa. Right Side

GROUP	1	2	3	4	5	6
Y	64	70	65	66	67	77
+	21	17	19	22	21	10
X	15	13	16	12	12	13
Total	100	100	100	100	100	100

Table VIIb. Left Side

GROUP	1	2	3	4	5	6
Y	80	79	68	81	73	79
+	14	12	19	9	21	11
X	6	9	13	10	6	10
Total	100	100	100	100	100	100

The maximum frequency of the Y pattern (81 per cent) is found in Group 4 (Table VIIb), while the minimum frequency of this pattern (64 per cent) occurs in Group 1 (Table VIIa). From this, too, it is obvious that a rational utilization of the figures is not possible, except by previous mathematical treatment.

Even though it would be tempting, with a view to comparative dental anatomy, to carry out a statistical analysis of the figures contained in Tables IV through VI, this seems too rash when the previously mentioned factors of uncertainty are taken into account. Therefore, the tabulated material is presented only graphically. Such a representation will, in the present circumstances, reveal variation tendencies in a readily perceptible way, although it will not, any more than a thorough statistical treatment, furnish detailed information about variation differences between the racial groups under consideration.

*A special method of calculating similar statistical premises is employed by Bertil Roos in his work: Über Die Karies des Milchgebisses bei Kleinkindern (chapter 5) (Acta pædiat. 31: suppl. 1. Lund, 1944). A computation according to this more correct method would for Hellman's material give a significance limit between 72.0 and 84.9 per cent.

In the tables, the percentile frequency of Y-patterned and five-cusped lower molars is computed. In Fig. 2 the frequency of Y pattern is ordinate, while the frequency of five cusps is abscissa. All points below the diagonal line correspond to patterns with a higher percentile frequency of five cusps than

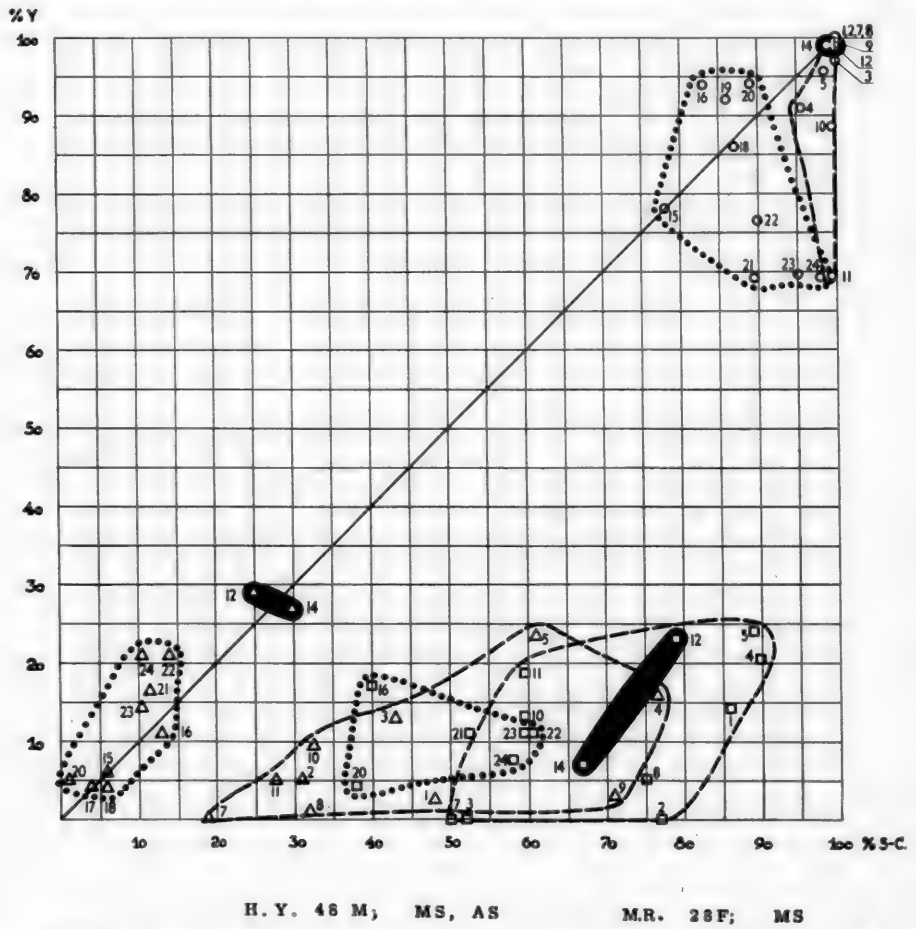

Fig. 2.—Correlation diagram showing the proportionate frequency of Y pattern and five cusps on the lower molars in different human populations (see Tables IV through VI). Circles: First molar. Triangles: Second molar. Squares: Third molar. The points for each of the lower molars in the major races are circumscribed with lines. Dotted line: The Caucasian race. Broken line: The Mongoloid race. Black area: The Negroid race.

of the Y pattern; reversely, all points above the line correspond to a higher frequency of Y pattern than of five cusps. The distribution of the points in Fig. 2 is conspicuous. One group of points, representing the first molar, is located in the right top corner, one group, representing the second molar of the Caucasian race, in the left bottom corner, while a large, very dispersed group, embracing the third molars of the Australoid and Mongoloid races, is

found along the axis of abscissa; the second molars of the Negroid race are found along the diagonal line, but separated from the second molar group of the Caucasian race.

The Caucasian race differs from the other races on the following points: (1) The first molar is relatively much modified; only on a single point (11: Texas Indians, 23 and 24: recent Danes) is there affinity to another race. (2) The second molar is very much modified; in regard to the cusp number, much more than the second molar in other races, but in regard to the groove pattern, to much the same extent as these. The group representing the second molars of the Causasian race is isolated. (3) The third molar: The frequency of the Y pattern almost coincides with M_2 in Caucasian and other races, and with M_3 in the other races. The frequency of five cusps is, on the whole, less than it is in other races. (4) From the fact that M_3 for Caucasians coincides with M_2 for Mongoloids, it may perhaps be inferred that M_2 in the Caucasian race used to have an occlusal pattern like that found in recent Mongoloids. (5) This points to a general tendency in man's second and third molars first to reduce the frequency of the Y pattern, and only later to reduce the cusp number essentially. (6) The Caucasian race is, merely on the basis of the relatively stronger modification of the occlusal patterns of the lower molars, well defined from other modern races.

The Mongoloid race (comprising Mongols, Chinese, Eskimos, and American Indians) is characterized as follows: (1) The dispersion of points for each lower molar is considerable. (2) The first molar: Only in a single representative (Texas Indians), is there an essential modification of the Y pattern. (3) The second molar is, with reference to the Y pattern, more strongly modified than it is in Negroes, but with reference to cusp number, almost equivalent to the Negroes. (4) The third molar is poorly defined from the second molar; only with regard to the cusp number, M_2 presents a somewhat stronger modification.

The Negroid race (African and American Negroes) is less represented in the present material than any of the aforementioned races. Until further, they may be characterized in this manner: (1) First molar barely modified. (2) Second molar: Y pattern and cusp number equally modified, but in a degree which isolates the race from other living races. (3) Third molar coincides with that of the Mongoloid race. (4) It is impossible on the basis hereof, and with the poor representation in mind, to tell in which of the two major Negro races the lower molars exhibit the strongest modification.

The Australoid race has presumably more primitive lower molars than the Caucasian race, but on the basis of the available data nothing definite can be concluded about its position in relation to the other races.

It appears from these data that the number of reliable conclusions based on the observations on the modification degree of parts of the Dryopithecus pattern referred to here is very small; the causes thereof have been stated previously.

The results so far attained indicate that a standardized, adequate investigation technique in this field would make it possible to show differences and similarities between the various ethnic groups and highly enlarge our knowledge of their interrelationship and origin.

SUMMARY

In the preface it is emphasized that in accordance with Gregory's original description, the Dryopithecus pattern of the lower molar teeth in simiinids and hominids is a complex of morphologic structures, whose evolutionary changes are little correlated phenotypically, and it is therefore suggested to treat them separately, both descriptively and statistically. This suggestion especially refers to the two structural "elements," the groove pattern and the cusp number. Further, it is suggested to divide the groove pattern into three well-definable, successive stages, indicated by the signs "Y," "+," and "X" (Fig. 1).

Material of recent Danish and Dutch lower molars is described (Tables I, II, and III). In the Dutch series there is a significant difference with regard to the frequency of the Y pattern on the right and left side first molars, and a considerable difference with regard to the cusp number on the right and left side third molars. Similar differences between the right and left sides could not be shown in the Danish series.

In discussing previous observations on the modification degree of the Dryopithecus pattern in the different ethnic groups, it is emphasized that a comparison on an exact statistical basis is rendered difficult by: (a) the absence of a precise definition of the concepts of groove pattern and cusp, (b) the different degree of wear in the specimens, and (c) the lack of a consistent distinction between right and left sides, and between males and females. Instead of discussing the material statistically, I have presented the results graphically (Fig. 2).

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A STUDY OF COPPER IN HUMAN ENAMEL

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COPPER was chosen for study because many observations suggest that its presence in teeth may be important. For instance, enamel with brown coloration has been found to have a high copper content.[1] This observation, together with the finding that trace amounts of copper in protein may catalyze enzymatic pigment formation,[2] suggests that the presence of copper may affect the coloring of teeth. There is also some clinical indication that high concentrations of copper in the enamel may produce resistance to dental caries.[1] Further support for this theory is provided by the laboratory findings that copper ions in concentrations of less than 5 ppm will exert an inhibitory effect on salivary acid production[3, 4] and that certain copper salts will reduce the solubility of powdered enamel.[5-8] However, with regard to the latter observation there is still some uncertainty, because no reduction in solubility was observed after application of a copper salt to intact enamel.[9]

It is interesting that of the tissues examined in a spectrochemical study bone was found to have the highest copper concentration.[10] The presence of copper in bone is undoubtedly associated with its function in blood formation, but it may have additional significance. This is suggested by the observations in animals that a deficiency in dietary copper may result in severe osteoporosis.[11, 12] There is, therefore, reason to believe that copper may play a role in bone formation, and it is possible that it may be an important factor in the calcification of teeth. It should be noted that copper has been found to be normally present in small amounts in saliva[4] so that a continuous pick up of copper by the enamel is possible.

Only a few attempts have been made to study the presence of copper in teeth. Lowater and Murray[13] observed that copper was a consistent tooth constituent. Sheldon and Ramage[14] found 0.002 per cent copper in a deciduous tooth and smaller amounts in six permanent teeth. Tiede and Chomse[15] estimated the copper content of human teeth to be in the range of 10^{-4} to 10^{-5} Gm. copper per gram tooth structure. Ginzburg and El'piner[16] found an average content of 5.2 γ of copper per gram of ash of sound teeth and 18.5 γ of copper per gram of ash of carious teeth. Rygge appears to be the only investigator who has studied the presence of copper in enamel, dentin, and cementum.[1] He found a copper content of approximately 0.01 per cent in the enamel of a tooth with pronounced brown discoloration, and only one-hundredth of this amount in the enamel of a normal tooth. No detectable

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copper was found in dentin or cementum. It is interesting to note that Rygge found the copper content of beaver enamel, which is naturally of a brown color, to be similar to that of brown human enamel.

It appears from the literature that copper commonly occurs in teeth, but that the concentration may vary considerably from tooth to tooth. Since no detailed report has been made of the presence of copper in the enamel, it was felt that a study of the distribution of copper in this structure would be of interest and that it also would be worth while to correlate the presence of copper in individual teeth with tooth appearance and tooth pathology.

METHOD

The following two procedures were used: (a) The copper content was determined in four successive layers of enamel obtained by grinding with a diamond stone. The ground enamel was collected in a porcelain crucible filter by means of suction, a procedure which brought the loss of enamel during grinding down to a minimum. The thinnest layers mechanically possible to grind were obtained from the outer surface to provide detailed information about the distribution of copper in the superficial enamel. Since we were interested in determining the amount of a number of trace elements* in addition to copper, the enamel from several teeth was pooled in order to provide sufficiently large samples.

Dental restorations and carious material were removed with a bur prior to the grinding, and the teeth were cleaned by gentle brushing with pumice and distilled water.

Enamel samples from four successive layers of fully formed impacted teeth and of erupted teeth from age groups under 20, 20 to 29, 30 to 49, and over 50, and from mottled teeth were included in the study. The ground enamel was dissolved in dilute hydrochloric acid and the volume was adjusted so that the concentration was 25 mg. of enamel per milliliter. A sample of 0.1 ml., therefore, containing 2.5 mg. was pipetted into the crater of a carbon electrode and dried. The electrode previously had received 100 Gm. of platinum in solution, the platinum serving as the internal standard. Special pure electrodes were used. The sample was burned as the negative electrode for three minutes, using a conventional DC arc method of excitation and a line potential of 130 volts and a current of 15 amperes. The spectrum was photographed, using a Bausch and Lomb medium quartz spectrograph, and the intensity was determined by means of a rotating sector method. Analyses were done in sextuplicate and the average was computed. Reagents were checked for copper contamination before use. Several runs were made on the dry enamel ash alone, the sample being simply weighed out and added to the electrode with nothing else. Spectrum lines measured were copper 3273.97 Å and platinum 2650.86 Å. The standard error of the results was 10 to 15 per cent.

(b) A different procedure was used in studying the copper content of the enamel of individual teeth because facilities were not available for accurately

*The presence of fluoride, lead, and tin in the enamel will be reported in separate papers.

weighing small amounts of enamel. Rather than grinding off and weighing enamel samples, the enamel was etched off the tooth by exposing the crown of the tooth under mechanical stirring to 8 ml. of 0.2M acetate buffer of pH 4 for 30 minutes. The acid exposure was repeated twice, each time in new aliquots of acid. Thus, three acid extracts, representing three successive layers of enamel, were obtained. Only sound enamel surfaces, including sound pits and fissures, were etched. This was achieved by covering carious lesions and restorations and the root portion with an impervious wax before the acid exposure. One ml. of the acid was used for phosphorus determination,[17] and the remaining 7 ml. were subjected to copper analysis according to the previously mentioned procedure. Since the copper analyses in these instances were done only in duplicate, the accuracy was somewhat less.

RESULTS

The findings on the ground enamel samples are shown in Table I. It is evident that copper is fairly evenly distributed in the enamel. There are small differences from layer to layer within each group, but no definite pattern of distribution. Somewhat lower concentrations were found in the enamel of impacted than of erupted teeth. However, there is no indication that the enamel will pick up copper, because the amounts found in the different age groups of erupted teeth were similar. The copper content of mottled enamel was slightly lower than that of normal enamel.

TABLE I

DISTRIBUTION OF COPPER (PPM) IN SUCCESSIVE LAYERS OF ENAMEL FROM THE SURFACE INWARD

LAYER NO.	UNERUPTED	ERUPTED				FLUOROSED
		UNDER 20	20 TO 29	30 TO 49	OVER* 50	
1	7	20	17	20	20	12
2	7	15	26	20	22	12
3	7	30	25	20	13	6
4	5	25	26	18	12	12

*Intact teeth only.

The fact that the copper level was found to be higher in the erupted than in the unerupted teeth led us to suspect that this difference in copper content might have been caused by contamination of the erupted enamel with copper-containing dental restorative materials. This possibility was checked by including only intact teeth in the over 50 years group. As will be seen from Table I, the copper values found in these teeth were similar to those of other erupted teeth. Therefore, there is no indication that contamination has affected our copper values.

The results obtained on individual erupted teeth are listed in Table II. Here is shown the amount of copper and phosphorus and the copper : phosphorus ratio of three successive acid extracts. The age and type of tooth and other data, including presence of brown pigmentation (BP) and white patches (WP), evaluation of surface roughness (1 to 3, from smooth to rough), and

TABLE II. COPPER AND PHOSPHORUS IN SURFACE ENAMEL—ERUPTED TEETH

TOOTH	AGE	TOOTH TYPE	COLOR CHANGES	SURFACE TEXTURE	FILLINGS	CARIES	FIRST ENAMEL LAYER CU (γ)	FIRST ENAMEL LAYER P (γ)	FIRST ENAMEL LAYER CU : P	SECOND ENAMEL LAYER CU (γ)	SECOND ENAMEL LAYER P (γ)	SECOND ENAMEL LAYER CU : P	THIRD ENAMEL LAYER CU (γ)	THIRD ENAMEL LAYER P (γ)	THIRD ENAMEL LAYER CU : P
1	7	E	B.P.*	1‡	no	yes	1.00	376	.00266	0.26	538	.00048	0.16	452	.00035
2	11	4		2	no	no	1.02	265	.00340	0.17	317	.00028	0.17	323	.00028
3	11	4		2	no	no	0.29	395	.00074	0.23	518	.00045	0.16	487	.00033
4	12	4		no data	no	no	0.11	354	.00032	0.09	326	.00028	0.09	317	.00029
5	13	4	W.P.†	no data	no	no	0.05	197	.00023	0.03	219	.00016	0.05	204	.00022
6	13	6	W.P., B.P.	2	yes	yes	0.38	316	.00120	0.19	414	.00046	0.21	490	.00043
7	15	4		no data	no	small	0.06	731	.00008	0.09	804	.00011	0.09	689	.00013
8	19	7	W.P.	2	no	yes	0.25	729	.00034	0.16	865	.00019	No data—for one hour		
8a§	19	8		2	no	yes	0.16	669	.00024	0.13	825	.00016	No data—for one hour		
9	21	8		2	no	yes	0.48	339	.00142	0.64	501	.00128	0.40	468	.00086
9a	21	7		2	no	yes	0.40	393	.00102	0.80	471	.00170	0.40	382	.00105
10	35	7		no data	no	no	0.14	173	.00080	0.14	143	.00097	0.14	125	.00112
11	38	8	W.P., B.P.	2	no	no	0.21	333	.00063	0.27	394	.00069	0.26	484	.00054
12	45	1	W.P., B.P.	1	one	one	0.32	273	.00117	1.60	378	.00424	0.64	391	.00164
13	45	1	W.P.	1	two	small	0.40	274	.00146	0.80	272	.00294	0.16	337	.00048
14	46	3	B.P.	1	no	no	0.27	365	.00074	none	389	–	0.00	404	–
15	52	2	W.P., B.P.	3	no	yes	0.16	180	.00089	0.15	216	.00070	0.13	128	.00103
15a	52	4	W.P., B.P.	2	no	yes	0.32	260	.00120	0.17	301	.00057	0.46	336	.00140
16	63	7	W.P., B.P.	1	one	yes	0.13	408	.00033	0.16	493	.00033	0.00	504	zero
17	77	5		1	one	small	0.29	275	.00106	0.25	361	.00069	0.00	321	zero
17a	77	4	B.P.	1	no	no	0.07	399	.00018	0.23	343	.00067	0.21	453	.00046
17b	77	4	B.P.	1	no	no	0.07	363	.00019	0.38	363	.00105	0.07	399	.00018

*B.P. denotes Brown Pigmentation
†W.P. denotes White Patches
‡denotes grades of roughness 1 - 3 from smooth to rough
§same numbers followed by a letter denote teeth from the same patient

TABLE III. COPPER AND PHOSPHORUS IN SURFACE ENAMEL—UNERUPTED TEETH

TOOTH	AGE	TOOTH TYPE	COLOR CHANGES	SURFACE TEXTURE	FIRST ENAMEL LAYER			SECOND ENAMEL LAYER			THIRD ENAMEL LAYER		
					CU (γ)	P (γ)	CU : P	CU (γ)	P (γ)	CU : P	CU (γ)	P (γ)	CU : P
1	15	8	W.P.	3	0.18	154	.00117	0.15	176	.00086	0.17	158	.00108
2	17	8	W.P., B.P.	2	0.23	480	.00048	0.16	660	.00024	0.16	664	.00024
2a	17	8	W.P., B.P.	3	0.35	517	.00068	0.80	664	.00121	0.39	758	.00052
2b	17	8	W.P.	2	0.29	537	.00054	0.80	549	.00146	0.80	642	.00125
3	18	8		3	0.25	482	.00052	0.29	530	.00055	0.21	539	.00039
4	20	8	W.P.	no data	0.41	108	.00380	0.12	121	.00099	0.08	120	.00067
5	22	8	B.P.	2	1.60	188	.00850	0.21	465	.00045	0.16	422	.00038

presence of fillings and caries are also tabulated. Copper was found in the outermost enamel of all the teeth. In one tooth, there was no detectable copper in the second etching, and in two other teeth, no copper was found in the third etching. The greatest amount of copper found in a single etching was 1.6 μg. The amount of phosphorus etched off the tooth was generally smaller in the first than in subsequent acid exposures, with values in different samples ranging from 125 to 865 μg. This variation in the amount of dissolved phosphorus in different etchings shows that different amounts of enamel went into solution in different acid exposures. Therefore, the acid extracts do not necessarily represent enamel layers of uniform thickness.

The copper : phosphorus ratio was sometimes greater (13 teeth) and sometimes smaller (8 teeth) in the first than in the second acid extract. The second acid extract generally had a larger copper : phosphorus ratio than the third (13 versus 5 teeth). The mean copper : phosphorus ratios of the three etchings were 0.00092, 0.00084, and 0.00054, respectively.

From the data in Table II, it appears that the copper : phosphorus ratio varies considerably in etchings from different teeth, undoubtedly reflecting differences in concentration of copper in the enamel. There is no relation between the copper : phosphorus ratio and age. Neither is there any correlation between the copper : phosphorus ratio and presence of brown pigmentation, white patches, surface roughness, fillings, or carious lesions.

Table III shows the data obtained on unerupted teeth. The results are similar to those of erupted teeth.

DISCUSSION

The copper concentrations found in the ground enamel samples closely agree with those observed in enamel by other investigators. Because they are also of the same order as those reported on bone, our findings strongly suggest that the copper present in the enamel is deposited during calcification and that no significant change in concentration takes place after eruption. If there were a posteruptive pick up, a considerable gain in copper might have been expected with age from the copper present not only in saliva and food, but also in certain chewing gums and dentifrices and many dental restoratives.

To obtain more direct evidence with regard to uptake of copper, the copper : phosphorus ratios of the enamel from four teeth from the same patient were determined. Two of these teeth were exposed to 0.05M and 0.005M of copper chloride, respectively, for 10 minutes, followed by washing in tap water for 10 minutes prior to the copper determinations. There was no appreciable difference in the copper : phosphorus ratios of the copper exposed teeth and the control teeth. Therefore, under these conditions, no copper was taken up by the surface enamel supporting the view that copper is not readily taken up after eruption. Of course, the possibility still exists that copper may react with plaque material on the tooth surface without actually forming a bond with the enamel.

The failure of copper to accumulate in the enamel shows that it reacts in a different way from fluorine and lead. Since many copper-containing pro-

teins are known to occur in animals and plants, it is likely that copper is associated with the organic part of the enamel and that it does not readily combine with the inorganic part under physiologic conditions. The reports in the literature that certain copper salts reduce the solubility of enamel might be taken to indicate that copper will react with the enamel, but in view of our findings, it is unlikely that this reaction represents an acquisition of copper by the enamel.

The copper : phosphorus ratios we found in the enamel etchings of individual teeth were frequently higher than might be expected. These high ratios could be caused by unusually high concentrations of copper in the enamel, but two other factors may be involved: (1) The copper concentration of 30 ppm was obtained from enamel of smooth surfaces and did not include pits and fissures. Our data from individual teeth represent pits and fissures as well as smooth enamel. Therefore, the possibility must be considered that the protein matter in pits and fissures may contain appreciable amounts of copper. (2) The copper and phosphorus values obtained on individual teeth represent the amount of these elements which went into solution in the acid. It is possible that copper leached out at a greater rate than did phosphorus.

The only certain conclusion which can be made from our observations on individual teeth is that copper occurred in appreciable amounts in the surface enamel of many teeth and that copper was readily etched from enamel at a pH of 4. This fact alone is of considerable interest because it raises the question as to whether copper ions may be released in sufficient concentration to interfere with the glycolytic cycle when acids are formed in the bacterial plaque on the tooth surface. Our data suggest that the amount of copper in the surface enamel may be great enough to be of protective value. The crucial question, as yet unanswered, is whether copper ions will go into solution at a sufficiently high pH to prevent dissolution of the enamel.

The theory that the presence of copper tends to render the enamel more resistant to acid is not supported by our findings. According to this theory, the enamel of teeth with a high copper content should have a low solubility and, therefore, give low values for dissolved phosphorus. A comparison of the copper and phosphorus values obtained from bicuspids and from third molars which had approximately the same area of enamel surface showed no such relation. However, it is possible that greater amounts of copper than is normally present in enamel may produce a solubility-reducing effect.

The lack of correlation of our copper data with presence of pigmentation, white patches, or caries does not rule out a possible significance of copper in the development of these anomalies, but, rather, points to the fact that many other factors may be involved. An allusion may be made to the well-established caries-inhibiting effect of fluoride and the failure to find a difference in the fluoride content of carious and noncarious teeth.[18] None of the teeth we examined had a deep brown color. Therefore, we were not able to check the finding of Rygge that this type of tooth is high in copper. The finding of Ginzberg and El'piner that the ash of carious teeth contains more copper than

the ash of noncarious teeth is also inconclusive with regard to a possible relation of copper to dental caries. It is possible that the high copper-to-ash weight ratio they found was caused by the decrease in the ash content of demineralized carious dentin without a corresponding decrease in the copper content. It is evident that much remains to be learned about the significance of the presence of copper in teeth.

SUMMARY

Copper was found to have a random distribution in depth in surface and subsurface enamel. The concentration in the external enamel layer averaged 20 ppm.

There was no appreciable difference in the copper content of the external enamel of erupted and unerupted teeth.

There was a wide variation in the copper content of the surface enamel from different teeth. No correlation was found between the copper content and enamel solubility, pigmentation, hypoplasia, or caries.

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THE LOCATION OF RADIOCALCIUM IN THE MANDIBULAR INCISOR AND ADJACENT STRUCTURES OF THE RAT

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ENAMEL matrix acquires a small mineral content almost as soon as it is deposited.[27] Subsequent to this limited, early calcification of the enamel matrix, an influx of minerals occurs in it until its final, highly calcified state is reached.[12, 24, 26] During the period of acquisition of minerals, enamel matrix undergoes a series of changes: 1. It becomes increasingly soluble in acids used to decalcify the specimen.[3, 4, 9] 2. Its staining characteristics are changed.[4] 3. It loses a portion of its moisture content.[7] 4. It changes from a homogeneous to a fibrillar structure when studied under the electron microscope.[31] 5. It exhibits altered birefringence when viewed with a polarizing microscope.[14, 21]

The progressive increments of mineral salts in enamel matrix have been demonstrated in various ways. Weinmann, Wessinger, and Reed[33] utilized chemical analyses to find that enamel, in progressing from its initial state to its final form, increases its inorganic content four times. Grenz rays were employed by Applebaum[1] to demonstrate that the development of radiopacity in enamel roughly paralleled the degree of mineralization of the tissue. Thewlis[12] developed a microphotometric method of measuring the percentage of calcific matter per unit volume of enamel and demonstrated an increase in the volume of the mineral deposit in the calcifying enamel of unerupted puppies' teeth. The density of enamel has been found by Deakins and Burt[8] to be correlated with its degree of mineralization, an over-all density increase of five times being observed during the completion of calcification of enamel.

A point of contention has existed concerning the source of the minerals which serve to complete the calcification of the enamel matrix. Two main pathways for the entrance of inorganic salts into the enamel matrix have been mentioned.[17] The centrifugal route implies the movement of substances from the pulp chamber to the enamel; the centripetal route assumes the passage of substances from the enamel organ into the enamel. There is evidence in the literature to support both views.

Beust[3] reported that the calcification of enamel matrix began after its deposition as a horny keratin-like material. He designated both the enamel organ and the pulp as sources of the inorganic salts.

Chase,[4] using decalcified sections, found it necessary to judge mineralization from changes occurring in the enamel matrix. He regarded the ameloblasts as the source of alterations observed. He found rhythmical modifications of the staining characteristics of the matrix which ceased, whether completed or not, three months after deposition.

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On the basis of their observations that the physical consistency of enamel matrix is hard, and that the enamel organ is reduced in size shortly after deposition of the matrix, Diamond and Weinmann[9] came to the conclusion that the total calcium salt content of completed enamel is present in the matrix as soon as it is laid down. They postulated that the calcium is present in a colloidal form and that the emergence of fully hardened enamel is brought about by a crystallization proceeding from the outer surface of enamel to the dentinoenamel junction. It is obvious that this hypothesis endorses the concept of the enamel organ serving as the source of calcium for the enamel matrix, but assumes an earlier time for its occurrence. The dilemma which this hypothesis sought to avoid, namely, the reduction in height of the ameloblastic layer before the completion of calcification, may be resolved in another way.

Irving[19] has shown that the presence of reduced ameloblasts does not necessarily result in a cessation of calcification of the enamel matrix. He found that by dietary means he could induce the formation of ameloblasts which were reduced in height. Although these cells could no longer elaborate new enamel matrix, that which was present was being calcified in a normal manner.

Karlström[20] demonstrated that the only difference in hardness of enamel before and after eruption occurred in the subsurface portions. He attributed this difference to a process of continuing mineralization of the deeper enamel by way of the pulp.

A histochemical study by Saunders, Nuckolls, and Frisbie,[27] later confirmed by Orban, Sicher, and Weinmann,[26] revealed that the very earliest enamel matrix is not calcified at all. The limited amount of calcification found in the enamel matrix before the more massive secondary calcification has begun enters first at the dentinoenamel junction and follows closely the deposition of the matrix. The technic used by these authors, however, is only applicable to the very early stages of enamel formation since it is based on the use of undecalcified sections.

In an interesting series of publications, Lefkowitz, Bodecker, and Shapiro[22, 23, 29] presented evidence that the calcification of the enamel matrix depends on the integrity of the odontoblasts. These investigators were able to prevent the histodifferentiation of fibroblasts into odontoblasts by surgical removal of the dental papilla of the tooth anlage. Degeneration of part of the enamel organ followed the operation, and the enamel matrix was not calcified beyond its initial state. These authors observed that even in areas where the reduced enamel epithelium had not undergone degeneration, no calcification of the matrix occurred. They therefore attributed the calcification of enamel matrix to the presence of the odontoblasts and regarded the calcification process as being of a continuous nature.

Wasserman[32] suggested that the pulp is the source of the inorganic salts which calcify the enamel matrix and that the enamel organ aids in the simultaneous removal of the organic matter and moisture from the matrix.

Radioisotope technics offer the possibility of locating newly acquired mineral salts. This in turn may suggest the route taken by inorganic materials during final calcification of the enamel matrix.

METHODS

Long-Evans female rats (used as controls in another study)[6] were raised from weaning on a purified diet* with a Ca:P ratio of 1 until they were 9 weeks old. They were then given an intravenous injection of 5 microcuries of Ca^{45} as $CaCl^2$. Sacrifice was performed under light ether anesthesia by decapitation at intervals of from one hour to eight days after administration of the radioisotope. Characteristic specimens were selected at the one-hour, two-day, and eight-day intervals. The mandibles were dissected free of soft tissues,

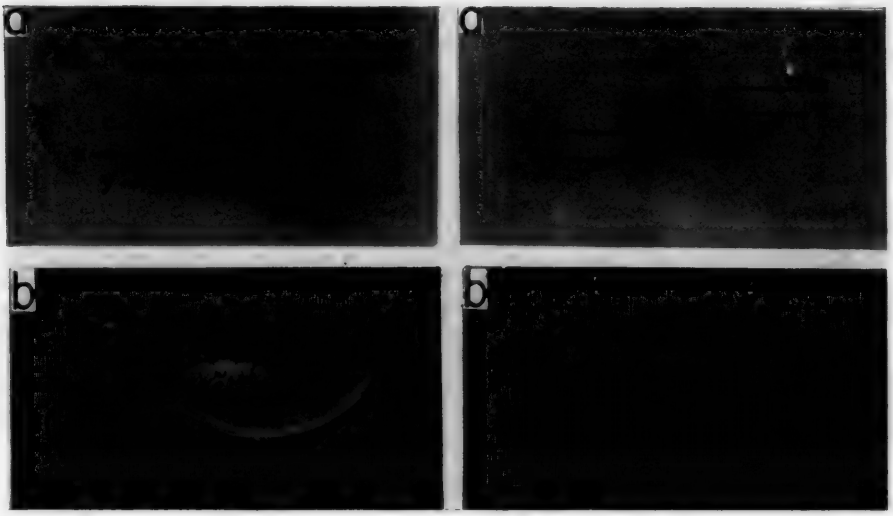

Fig. 1. Fig. 2.

Fig. 1.—*a*, Radioautograph-roentgenogram of a mandible of a rat sacrificed one hour after administration of Ca^{45}.
m: Blackening at the junction of pulp and dentin of molars.
i: Blackening at the junction of pulp and dentin of incisors.
c: Blackening at lamina dura.
x: Blackening in region of odontogenic epithelium.
y and *z*: Blackening at labial surface of roentgenographically opaque enamel. (×3; reduced ¼.)
b, Roentgenogram of same section as in Fig. 1, *a*. Code is the same. (×3; reduced ¼.)

Fig. 2.—*a*, Radioautograph-roentgenogram of mandible of rat sacrificed one hour after administration of Ca^{45}. Code and enlargement same as in previous figures.
b, Roentgenogram of same section as in Fig. 2, *a*.

fixed in dioxane, embedded in plastic, and sectioned as described previously.[25] Radioautographs were made from the mandibles and their accompanying teeth using Industrial Type A x-ray film and an exposure of one and one-half months. Roentgenograms of the sections were also obtained, both before and after removing the specimens from the radioautograph.[25]

Each of the illustrations presented (Figs. 1 to 4) has two parts. Part *a* is a radioautograph superimposed on a light roentgenogram of the section to aid the orientation. Part *b* is a plain roentgenogram of the same section to demon-

*Fibrin (Armour washed) 25 per cent
Elvehejm salt mix 4 per cent
Water-soluble vitamins 1 per cent
Powdered sucrose 60 per cent
Fat 10 per cent
Fat-soluble vitamins 1 per cent

strate the density of the various tissue components. The black areas of part *a* of each figure are produced by the soft beta radiation of Ca^{45}. The roentgen rays have produced no effect darker than the background seen in each radioautograph, so their effects can be differentiated from those of the radioisotope. In part *b* of each figure the interpretation may be routine since these are ordinary roentgenograms of the same sections. No attempt will be made to describe part *b* of each figure. Reference will be made to it only to relate the sites of uptake of Ca^{45} to roentgenographic features.

Figs. 1 and 2 are from animals sacrificed one hour after the administration of the isotope. Figs. 3 and 4 are from specimens which received radiocalcium two and eight days, respectively, prior to sacrifice.

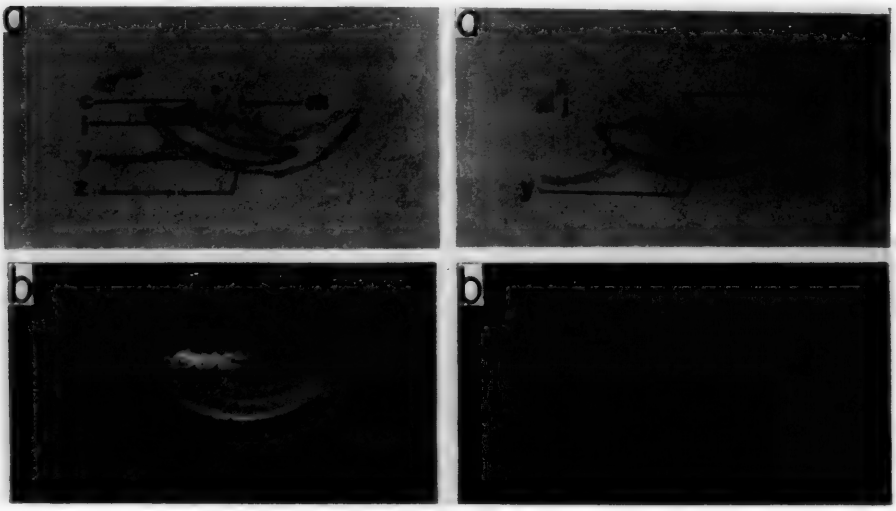

Fig. 3. Fig. 4.

Fig. 3.—*a*, Radioautograph-roentgenogram of mandible of rat sacrificed two days after administration of Ca^{45}. Code and enlargement same as in previous figures.
b, Roentgenogram of same section as in Fig. 3, *a*.

Fig. 4.—*a*, Radioautograph-roentgenogram of mandible of rat sacrificed eight days after administration of Ca^{45}. Code and enlargement same as in previous figures.
b, Roentgenogram of same section as in Fig. 4, *a*.

FINDINGS

The chief sites of blackening in the radioautographs may be summarized as follows: 1. A thin ring demarcating the periphery of the pulps of the molars (*m*) and a thick band in an equivalent position in the incisors (*i*). 2. A heavy band along the cortical plate of the alveolar process and a similar band at the posterior border of the section (*c*). 3. An irregular zone located at the height of the condylar process whenever this latter structure is included in the section (*j*). These findings will not be repeated for each description inasmuch as they are common to all the illustrations. Instead, detailed analyses will be confined to the location of Ca^{45} in the enamel of the incisors, since this subject is the prime concern of this paper.

One hour after the administration of Ca^{45} to the animals, the isotope may be found at the growing end of the incisor teeth. This may be seen in the radioautograph of Fig. 1 made from a fairly central section through the incisor. The pulp of the incisor is outlined by the blackening produced by Ca^{45}. At *x*, an area of reduced silver is found which is separate from the blackening in the pulpal portion of the tooth. It is of a size to preclude its being confined to either the tapered thin layer of calcified tissue characteristic of this end of the incisor or to the ameloblastic layer. A roentgenogram of the section (part *b*) indicates the absence of a roentgenopaque structure at *x*.

The presence of Ca^{45} is also found at *y* in the radioautograph of Fig. 1. These blackened areas are elongated and flattened in comparison to the one found apically. They are located approximately beneath the first and second molars at points opposite radiopaque structure of the incisor. The roentgenogram (part *b*) does not make clear whether or not these areas are actually within the enamel or merely alongside it.

A section lingual to the one used as Fig. 1 is represented in Fig. 2. The incisor is cut in a decidedly oblique direction and its extreme apical end is not represented. The radioautograph again shows the location of Ca^{45} one hour after its administration. A blackened elongated area occurs at the labial aspect of the incisor at *y* and is distinctly separated from the pulpal accumulation of Ca^{45}. The roentgenogram (part *b*) indicates that the radiocalcium is located in a radiolucent zone which has also been designated as *y*.

Two days after the intravenous injection of Ca^{45}, the enamel of the incisor contains the isotope chiefly in its central third (Fig. 3). Two discrete zones of blackening are indicated at *y* and *z*. The more apical of the two is also the heavier, and its separation from the pulpal component of Ca^{45} is no longer sharply delineated. In the roentgenogram (part *b*), it is within a portion of the incisor which is radiopaque. The more anteriorly located Ca^{45} (*z*) is still clearly separate from the pulpal blackening. This separation is responsible for some uncertainty when attempts to localize *z* in the roentgenogram are made.

Fig. 4 shows the location of Ca^{45} in the incisor of an animal sacrificed eight days after receiving the radioelement. In the radioautograph a large part of the enamel in the central third of the incisor shows heavy blackening. The section passes obliquely through the incisor pulp chamber, and that portion of the pulpal radiocalcium which is present blends imperceptibly with that of the enamel *y*. In the portion of the incisor anterior to the pulp, the enamel Ca^{45} takes the form of a tapering band. Apically, the enamel Ca^{45} and that of the pulp are indistinguishable, there being no line of separation. A black area in the center of the erupted part of the incisor corresponds with the presence of the residual pulp in this location.[28] The roentgenogram (part *b*) provides a guide for determining the relative location of the blackened regions.

DISCUSSION

Since only one hour had elapsed from administration of the Ca^{45} to the first interval of sacrifice, it is probable that the appearance of the labeled ele-

ment in Figs. 1 and 2 reflects only an exchange or adsorption rather than apposition of new tissue.[11] It is probable that the sites of intensive calcification have a high degree of accessibility to body fluids.[15] This would also bring about an increased rate of exchange or adsorption of isotopes in these areas. Although the presence of Ca^{45} does not imply a deposition of new skeletal tissue, it is also apparent that the radioelement is not associated with the incisors and molars only during the short period of high concentration in the plasma. This is shown in Fig. 4, which clearly demonstrates blackening due to Ca^{45} in the incisor and bone eight days after administration of the isotope. The blood level of Ca^{45} at this point is low enough to be negligible.[6] We may conclude that the Ca^{45} seen earlier has not been lost to any significant degree by continued exchange with the plasma Ca^{40}. Fig. 4 also indicates that the Ca^{45} has moved anteriorly with the continued eruption of the incisor.

Figs. 1 and 2 demonstrated that the Ca^{45} found at the labial portions of the incisor during the first hour is separate from that entering the tooth through the pulp. It is significant that blackening in the region of the enamel is usually found apical to a perpendicular dropped from the first molar and parallel to its long axis. Kitchin[21] designated this anatomic landmark as the site of the first appearance of crystallization as judged by polarization microscopy. D'Iorio and Lussier[10] found the greatest uptake of Ca^{45} by portions of the dental apparatus which had less than maximal density. This fraction would include enamel which was still incompletely calcified, such as is found in the apical half of the incisor.

Of further interest is the finding that roentgenographically opaque, as well as transparent, enamel is associated with sites of Ca^{45} uptake. The presence of mineral salts in young enamel matrix,[16, 27] and in the ameloblasts as well,[18] has been reported previously. The appearance of Ca^{45} in or close by to both radiolucent and radiopaque enamel suggests a continuous entry of calcium into enamel during a considerable part of its pre-eruptive phase. Such calcium acquisition appears to have no physical connection with the dentinal component during the early phases. After eight days a continuity between dentinal and enamel Ca^{45} is established.

A consideration of the first two illustrations suggests that during the first hour after injection Ca^{45} is taken up in areas where calcified tissues are being newly formed. The apical end of the incisor has long been established as an actively calcifying region,[2, 30] as has the condyle of the mandibular ramus.[5, 30] Dentin formation has been observed in molars for periods twice as long as the age of the animals used in this study.[18] Sicher and Weinmann[30] have reported a continuous vertical movement of the molars of the rat which compensates for attrition of these teeth and growth of the body of the mandible. This implicates the alveolar process as a site of growth. The presence of a heavy accumulation of Ca^{45} in the cortical alveolar bone is a by-product of such activity.

The blackening at the posterior border of the sections (the lingual cortical plate of bone) is probably due to calcifying activity at this site also. The molar teeth drift distally concomitant with new apposition of bone mesial to them.[30]

Due to the lingual inclination of the mandibular molars and their supporting bone, the distal termination of the alveolar process juts out lingually from the mandible in the form of a ridge. The posterior surface of this ridge, which is also part of the lingual surface of the mandible, must receive new apposition of bone during the distal migration of the molars. This ridge forms a part of the posterior border of the sections studied.

SUMMARY AND CONCLUSIONS

1. The chief sites of Ca^{45} uptake in the mandibles of rats one hour after its administration are the same ones which are known to have active deposition of mineral salts occurring.

2. One hour after intravenous administration, Ca^{45} found at the labial enamel of the incisor is distinctly separate from that found in the dentin.

3. Ca^{45} is found within or immediately adjacent to both radiolucent and radiopaque portions of the developing enamel.

4. The Ca^{45} found in the vicinity of the pulp and that found in the labial portion of the tooth fuse at some time from two to eight days after administration of the isotope.

5. Radiocalcium is found in the teeth and bone one week after the blood level of this isotope is no longer favorable to exchange reactions with these tissues.

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THE COMPARATIVE TURNOVER RATE OF P^{32} IN NORMAL AND DEFICIENT RATS' TEETH AND BONES DURING RECOVERY FROM PHOSPHORUS DEFICIENCY

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IN A previous paper,[9] the mineral ash contents of the femurs, incisors, and molars of rats during recovery from phosphorus deficiency were given. It was found that relative to the gain in body weight, the femur ash increased mostly during the second week of recovery while tooth ash did so only during the first week. It was also observed that failure to demonstrate chemical changes in the teeth was due to the small total volume subject to alterations. The present paper is a supplement to the previous one and reports the results of a radiochemical analysis of the response of teeth and bones of rats to recovery from phosphorus deficiency.

Studies with radioisotopes of Ca and P have already been applied to the problem of rickets in experimental animals and its subsequent healing. Dols, Jansen, Sizoo, and Barendregt found no differences in the radiophosphorus content of lipids of phosphorus-deficient, normal, or vitamin D-treated rachitic rats.[4] They also were unable to observe any changes in the amount of phosphorus re-excreted into the intestine by these same types of animals.[3] The rachitic rats of Cohn and Greenberg[2] had an increase of P^{32} in their bones of 25 to 50 per cent when given vitamin D. The total increase in phosphorus was mostly at the expense of the newly ingested phosphorus.

According to Morgareidge and Manly,[8] the first appearance of P^{32} in the rachitic metaphysis following the administration of vitamin D occurs at the same time as the earliest appearance of the positive line test. Gaunt, Griffith, and Irving[5] fed rats on diets low in both Ca and P. These animals retained more P^{32} than the control group, most of the difference occurring in the muscles. Our own calculations made from data they presented reveal the following specific activity findings: The deficient animals' bones had 15 per cent and the incisors 8 per cent greater specific activities than the controls. The molar specific activities of the controls, however, were 5 per cent higher than those of the deficient animals.

The effect of recovery from phosphorus deficiency on the intestinal excretion of phosphorus was studied by Kjerulf-Jensen.[6] Restoring phosphorus to the diet produces an increase in the endogenous phosphorus of the gut, which was explained by postulating the existence of an exchange reaction occurring in the intestinal wall.

Migicovsky and Emslie[7] found the specific activity of chick tibias, from orally administered Ca^{45}, to be greater for rachitic chicks given vitamin D than

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for the untreated ones. Intramuscular administration of Ca^{45} resulted in higher specific activities of the untreated animals' tibias.

In Carlsson's[1] study of phosphorus-deficient rats, the incisor uptake of Ca^{45} was unaltered by either deficiency or recovery. The femurs showed severely retarded abilities to acquire Ca^{45} during the deficiency. Vitamin D brought about more normal metabolism in the femurs. In addition, during recovery the incisors exhibited no loss of Ca^{45} due to resorption as did the femurs.

Differences between the incisors and femurs of normal, recovering, and rachitic rats were also reported by Underwood, Fisch, and Hodge.[10] Incisors responded to vitamin D administration with an immediate rise in Ca^{45} content, while femurs exhibited a delay prior to any such increase.

METHODS

The experimental arrangement and schedule of sacrifice are the same as reported earlier[9] except for the intraperitoneal injection of 2 microcuries of P^{32} as a carrier-free isotonic solution. One-half the animals were injected at the beginning of the first week of recovery and the remainder received P^{32} at the start of the second week. The femurs, incisors, and molars of the left sides of the animals were dissected away for analysis. The specimens were fat-extracted, ashed, radioassayed, and analyzed for total inorganic phosphate by the colorimetric method of Fiske and Subbarow. Radioassay was performed in one-inch bottle cap planchets, the ash of the specimens being deposited in them by evaporation of the solvent. A Tracerlab Autoscaler with a Geiger tube having a window thickness of 2 mg. per square centimeter was employed.

TABLE I

RATIOS OF SPECIFIC ACTIVITIES OF RECOVERING PHOSPHORUS-DEFICIENT ANIMALS' FEMURS, INCISORS, AND MOLARS TO THOSE OF THEIR CONTROLS

	RATIO OF P-DEFICIENT SPECIFIC ACTIVITY TO THE AD LIBITUM CONTROLS			RATIO OF P-DEFICIENT SPECIFIC ACTIVITY TO THE PAIR-FED CONTROL		
DAYS OF RECOVERY DIET	FEMURS	INCISORS	MOLARS	FEMURS	INCISORS	MOLARS
0	7.7	1.8	2.0	5.2	1.4	1.2
1	8.7	2.0	2.3	4.0	1.5	1.3
2	6.6	1.2	2.1	3.7	0.98	1.3
3	9.8			6.7		0.93
4	8.0	1.8	2.0	3.6	1.4	1.0
6	4.9	1.8	2.8	3.2	1.2	1.3
7	5.1	1.6	1.9	3.2	1.4	1.2
	Second Week of Recovery					
8	16.2	1.2	1.9	4.9	1.3	1.2
10	13.0	1.7	1.4	3.2	1.0	1.3
12	10.5	1.1	2.4	3.9	1.2	1.3
13	10.5	1.1	2.0	4.0	0.95	1.5
14	12.8	1.3	2.3	3.6	1.1	1.9
Mean ratio	10.8	1.52	2.10	4.1	1.3	1.4
Standard deviation	3.84	0.583	0.729	1.61	0.37	0.59
Standard error of mean	1.05	0.185	0.231	0.49	0.12	0.13

Corrections were made for decay with the aid of a specially prepared P^{32} standard. Self-absorption was compensated for with a curve based on a series of determinations on tricalcium phosphate.

Data were calculated as a percentage of administered dose per milligram of inorganic phosphorus. The specific activity values so obtained were compared, and the result of this was expressed as a ratio of the phosphorus-deficient animals' scores to those of the ad libitum and pair-fed controls.

FINDINGS

The ratios of the specific activities are presented in Table I. The values were obtained by dividing the ad libitum animals' specific activities into those of the phosphorus-deficient ones. In Fig. 1, the mean value (taken from Table I) of the entire fourteen-day period is indicated by the horizontal line.

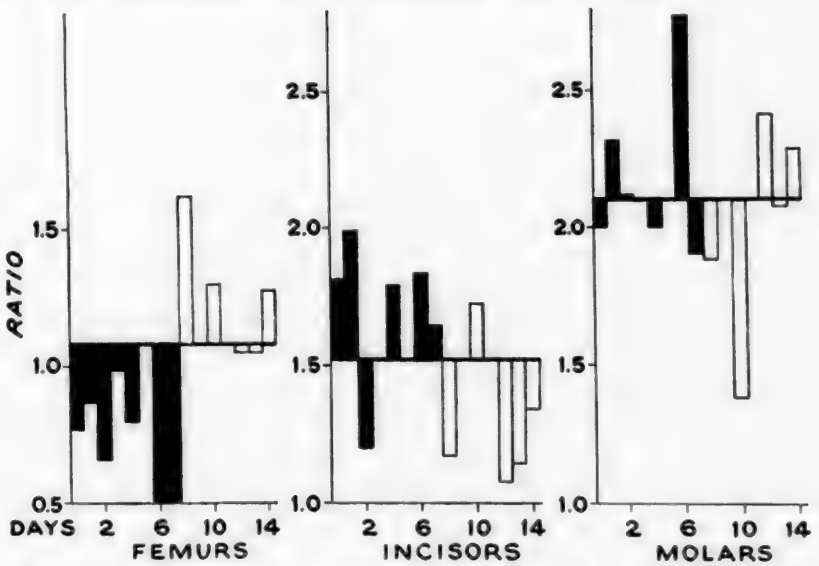

Fig. 1.—Ratios of recovering phosphorus–deficient animals' specific activity to that of the ad libitum–fed controls for femurs, incisors, and molars, over two successive weeks of recovery.

The daily fluctuations from the mean are depicted by the length of the bars in each graph. Part *A* indicates the response of the femurs during the first week of recovery (black column) and during the second week (white column). The femur specific activity ratios of the first week of recuperation are consistently smaller than the mean value. The second week generally has scores above that of the mean. At the twelfth and thirteenth day, two exceptions to this rule occur. However, even these values are higher than any of the scores during the first seven days of recovery. Parts *B* and *C* present the ratios for the incisors and molars and exhibit a random distribution of the daily scores about the mean. The ratios of the second week of recovery are not appreciably different from those of the first week in the case of the teeth.

A graph of the ratios of the phosphorus-deficient to pair-fed control animals' specific activities was made. In general, all three parts of this graph (incisors, molars, and femurs) resembled those of the previously mentioned series. It is not presented here.

A comparison of the mean ratios present in Table I is revealing. The greatest difference between specific activities of the ad libitum and p-deficient rats is found in the femurs and is a factor of 10. The phosphorus-deficient incisors' specific activity averages 1.5 times more than that of the ad libitum–fed controls, while the molar value is about 2. The pair-fed control animals' specific activities show a ratio of 4 for the femurs, and 1.3 and 1.4 for the incisors and molars when divided into the phosphorus-deficient scores. Table I lists the standard deviations and standard errors of the mean. From it the ranges may be calculated within which differences between the observed and true mean will be found 95 per cent of the time.

DISCUSSION

If the specific activities of the control and experimental groups were the same, the ratio between them would approximate a value of 1.0. Such a result is found in the incisors and molars. The question arises as to whether or not the observed ratios for the teeth may be regarded as significantly greater than 1.0. From the values in Table I it may be calculated that the mean ratio for the fourteen days plus or minus two times the standard error of the mean yields values which are still greater than 1.0. We may conclude that in any series of observations of equal length, variability, and magnitude as those of the ratios, the value of 1.0 would be a rare occurrence. The recovering animals' teeth may therefore be regarded as exhibiting a slightly but significantly greater specific activity than those of the controls.

In the case of the femurs, the ratio is appreciably greater than 1.0. This again is due to the larger magnitude of the recovering animals' specific activity compared to that of the controls. The difference is at its maximum during the second week of recovery, a fact which is consistent with the idea that the rachitic osteoid must first be resorbed and new matrix laid down before extensive repair can occur in bones.

Differences of lesser magnitude without maximal values during the second week were seen when comparisons were made of femur specific activities of recovering and pair-fed animals.

It is of interest to point out that the ratios between the specific activities of the teeth are not at their maximal value during the second week of recuperation. In fact, no maxima can be distinguished anywhere during the two-week period. This observation also conforms with the concept that teeth bear a permanent record of disturbed mineralization. No resorption of uncalcified tooth matrix occurs, the results of recovery are simply superimposed over the older unmineralized matrix. This may explain why the difference of specific activity in recovering and control animals' teeth manifests itself immediately in contrast to a delay in the case of the long bones.

SUMMARY AND CONCLUSIONS

1. At the end of the first fourteen days of recovery from phosphorus deficiency the femurs of the phosphorus-deficient animals have a ten times greater turnover rate of P^{32} than those of the ad libitum controls. Most of this difference is created during the second week of recovery.

2. Incisors and molars of recovering phosphorus-deficient rats have only slightly higher (greater than one but less than two times) turnover rate than those of their controls. The magnitude of this difference is not noticeably influenced by pair feeding or by the degree of recovery which occurs during the first weeks of eruption.

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THE CORRELATION OF GAIN IN BODY WEIGHT AND MINERAL ASH CONTENT OF TEETH AND BONES OF RATS RECOVERING FROM PHOSPHORUS DEFICIENCY

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THE first fourteen days of recovery which follow phosphorus deficiency are characterized by the most intensive series of changes of the entire experimental period. Gross signs of recovery such as gain in weight, improvement of coat texture, disappearance of hemorrhagic exudates from the eyes and snout, and steadying of the gait of the animal can all be observed in an interval of fourteen days.[11] Histochemical and morphologic studies made during this period confirm the impression of precipitous recovery.[19, 22] Since phosphorus deficiency has such a marked influence on the mineral metabolism of the rat, it is to be expected that skeletal structures such as the bones and teeth are subject to notable variations in composition. Much has been reported on the bone changes in low-phosphorus rickets. Surprisingly poor correlation has been found, however, between the mineral content of bones and that of teeth when animals are subjected to phosphorus deprivation. Early studies, such as those of Gies and Perlzweig,[10] which reported chemical aberrations of the teeth due to phosphorus deficiency, are undoubtedly of limited value because of the use of nonpurified diets. Karshan and Rosebury[12-14] found a 50 per cent decrease in bone minerals of rats on a low-phosphorus ration but no corresponding reduction in the teeth. Their findings have been confirmed by Mullick,[20, 21] Gaunt and Irving,[8, 9] and Follis, Day, and McCollum.[7]

It seems incongruous that in young animals only the bones should exhibit significant degrees of hypocalcification. This is especially so in view of the histologic demonstration by Becks and Ryder,[1] Coleman, Becks, Copp, and Frandsen,[3] and Rosebury and Karshan[23] that wide dentinoid seams are present in the teeth of phosphorus-deficient rats. Even if it is recognized that bones are likely to be more profoundly affected than teeth by disturbance of mineral metabolism, it is still difficult to correlate the morphologic and chemical findings in teeth.[15, 20, 21]

The intensive reparative process which follows the restoration of phosphorus to a diet low in that element provides an excellent vehicle for analyses of the alterations which occur in teeth and bones. The accelerated reactions during the first two weeks of recovery might be expected to exaggerate the repair of tissue changes caused by the preceding period of deficiency.

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METHODS

Ninety female Long-Evans rats were placed on an adequate stock diet at 21 days. Three to four days later the animals were divided into three experimental groupings. The first group received a purified low-phosphorus ration with a calcium:phosphorus ratio of 4. The second group received a purified normal control diet with a calcium:phosphorus ratio of 1 and was pair-fed to the deficient animals. The remainder of the animals were fed the control diet ad libitum.* All animals were weighed every two days.

At 72 days of age the deficient animals were taken off their rachitogenic diet and given the control ration. Sacrifice was accomplished during the next fourteen days, two animals from each group being decapitated at each interval. The femur and mandible of the right side were dissected free of soft tissue, and the mandible was dissected to yield the three molars and the incisor. The long bones were weighed, fat extracted with hot Bloor's reagent, dried to constant weight, and then dry-ashed at 650° C. The ash was subsequently dried to constant weight.

The animals sacrificed on the same day as change-over of the diets also yielded the femur, incisor, and molar specimens from the left side, which were decalcified, sectioned, and stained with hematoxylin and eosin.

Data were derived as follows: The average body weight of the animals in grams on each day of sacrifice was divided by the average ash weight of the femur in milligrams. In this manner a ratio is obtained which serves to compare mineral content changes to a standard of reference, the body weight. The incisor and molar ash weights were treated similarly.

FINDINGS

Fig. 1 is the composite growth curve of the three groups of animals used in this study. The deficient animals show a retarded rate of growth during the time they were on the low-phosphorus diet.[6] The substitution of the control diet for the deficient one is promptly followed by a rapid gain in weight. The pair-fed animals parallel the growth of the deficient animals from the time of initiation of pair-feeding to the date of sacrifice. During the recovery period the deficient animals ate increasing amounts of the ration. This is reflected in the increased growth rate of the deficient rats and their pair-fed controls. The ad libitum-fed controls gained weight rapidly at first. The rate of gain diminished during the later stages of the seven-week growth period. The gross symptoms in general were similar to those reported by Day and McCollum.[5]

Figs. 2, 3, and 4 present graphs of the body weight: ash weight ratios for the femurs, incisors, and molars. Each illustration is composed of three parts: the graphs for the recovering deficient animals, those for the pair-fed controls, and finally those for the ad libitum controls. Each graph may be examined for

*25% Armour washed beef blood fibrin
4% Salt mix (Elvehejm)
1% Water-soluble vitamin mix
1% Fat-soluble vitamin mix
10% Vegetable shortening
60% Powdered sucrose
The low-phosphorus diet substituted KCl for K_2HPO_4 in the control ration.

the following information: (1) The mean value of body weight divided by ash weight for the entire fourteen-day period. (2) The magnitude of the daily variations of the ratios which go to make up the mean. (3) Any trends in the variations from the mean, such as high ratios early in the fourteen-day period and low ratios later on.

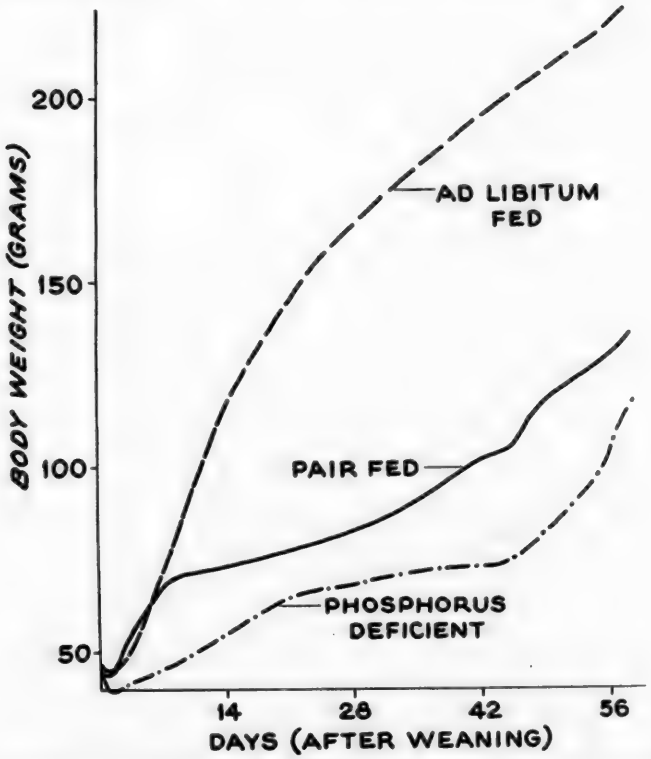

Fig. 1.—A composite growth curve of ad libitum, pair-fed, and deficient animals from weaning until sacrifice.

Fig. 2 presents the ratios of body weight in grams to ash weight in milligrams for the femurs during the fourteen-day interval of healing. Bar graph *A* indicates the daily variations of the ratios from the mean value of the entire fourteen-day period (horizontal line) for the recuperating animals. During the first four days the ratios are greater than the mean, but during the remaining ten they are smaller. The ratios thus show a distinct trend toward lower values during later stages of recovery from phosphorus deficiency.

Similar trends cannot be identified for the pair-fed and ad libitum-fed rats (bar graphs *B* and *C*). The scatter of the ratios around the mean is random and does not depend on whether the observation is made early or late in the recovery period.

Fig. 3 contains three bar graphs of the ratios of body weight to ash weight of the incisors. The distribution of the values of the recovering animals' in-

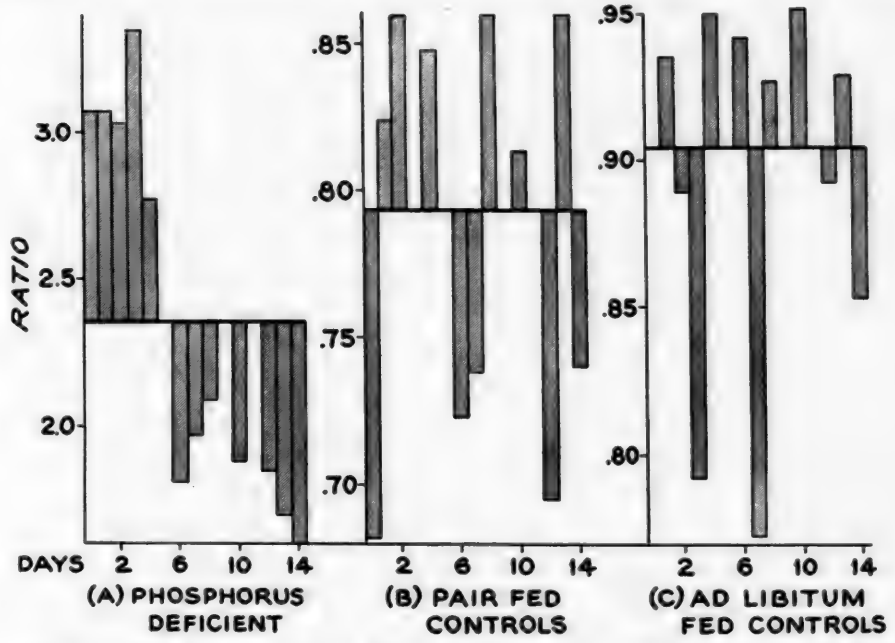

Fig. 2.—Bar graphs *A*, *B*, and *C*, depicting the daily variation of the ratio of body weight to ash weight of femurs in recovering phosphorus-deficient, pair-fed and ad libitum-fed rats.

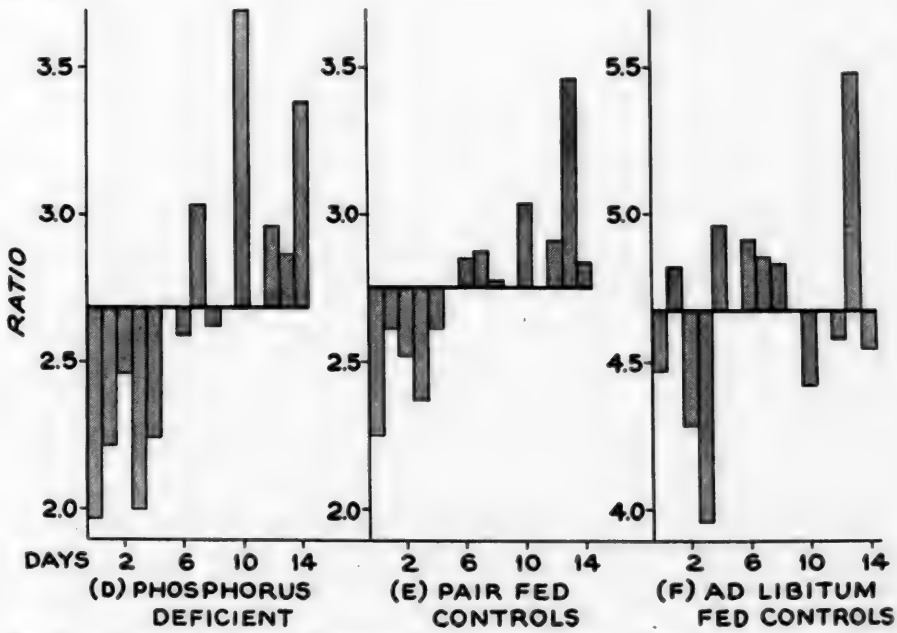

Fig. 3.—Bar graphs *D*, *E*, and *F*, depicting the daily variations of the ratio of body weight to ash weight of the incisors in the three aforementioned groups.

TABLE I*

STATISTICAL ANALYSIS OF AVERAGE RATIOS OF BODY WEIGHT TO ASH WEIGHT OF FEMURS, INCISORS, AND MOLARS FOR THE FOURTEEN-DAY HEALING PERIOD

	FEMURS			INCISORS			MOLARS		
	PHOSPHORUS-DEFICIENT	PAIR-FED CONTROL	AD LIBITUM-FED CONTROL	PHOSPHORUS-DEFICIENT	PAIR-FED CONTROL	AD LIBITUM-FED CONTROL	PHOSPHORUS-DEFICIENT	PAIR-FED CONTROL	AD LIBITUM-FED CONTROL
Mean ratio of 14-day period	2.35	0.793	0.904	2.68	2.76	4.67	3.87	4.63	7.55
Standard deviation	0.959	0.251	0.281	0.969	0.889	1.48	1.34	1.53	2.30
Standard error of mean	±0.290	±0.0759	±0.0850	±0.293	±0.269	±0.447	±0.405	±0.462	±0.695
Probability of difference between phosphorus-deficient and ad libitum, means being due to chance		Less than 0.01			Less than 0.01			Less than 0.01	
Probability of difference between pair-fed and ad libitum, means being due to chance		Greater than 0.5			Less than 0.01			Less than 0.01	

*Probability values are derived from Snedecor's table of "t":

$$\text{"t"} = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\left(\frac{N_1 \sigma_1^2 + N_2 \sigma_2^2}{N_1 + N_2 - 2}\right)\left(\frac{N_1 + N_2}{N_1 N_2}\right)}}$$

cisors (bar graph *D*) around the mean score for the period as a whole is opposite that found for the femurs. The ratios are lower than the mean during the first part of the two-week period and higher during the latter part. Almost the same pattern occurs for the pair-fed controls' incisors (bar graph *E*). The ad libitum-fed animals (bar graph *F*) show no differences in the pattern of ratio scores either early or late in the experimental period.

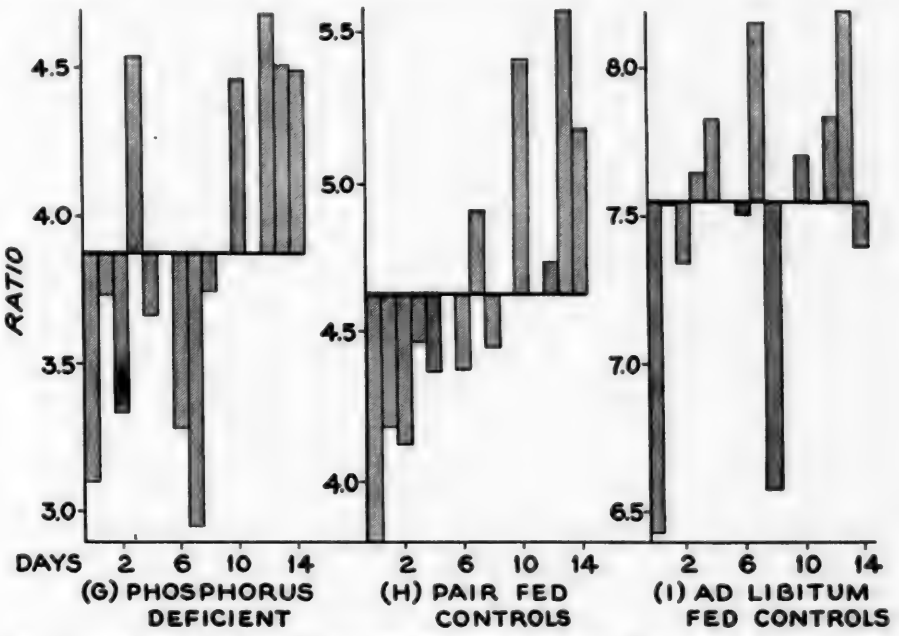

Fig. 4.—Bar graphs *G*, *H*, and *I*, depicting the daily variations of the ratio of body weight to ash weight of the molars in the three experimental groupings.

The molars have been analyzed similarly to the incisors and femurs, and the results are found in Fig. 4. Once again the early period of recovery is characterized in both P-deficient and pair-fed animals by ratios which are generally less than the mean (bar graphs *G* and *H*). During the latter part of the experimental period the daily ratios exceed the mean. A random distribution of the ratios for the ad libitum-fed rats is indicated in bar graph *I*.

A comparison of the means is also of interest. It will be seen from Table I that the mean ratios of incisors and molars of the ad libitum-fed rats are about two times those of the experimental and pair-fed controls. The body weight:ash ratios of the femurs of the ad libitum animals, however, are less than one-half the ratios of the experimental group and about equal to those of the pair-fed controls.

Table I also provides a statistical analysis of the differences in the mean ratios of the three different groups of animals. Of note is the finding that chance plays a very small role (1 per cent) in establishing the difference between the

mean ratios of the ad libitum-fed and the other two groups of animals. The sole exception occurs in the femurs, where no significance can be attributed to the difference between the mean values of the ad libitum-fed and pair-fed controls.

DISCUSSION

The use of ratios in comparing ash and body weight validates the following general rules of interpretation: (1) If the ratio increases during the two-week period, it is because the gain in weight of the animals has increased at a faster rate than the gain in mineral ash of the tissue being studied. (2) Contrariwise, if the ratio declines, the ash weight has increased more rapidly than the body weight. (3) If the ratio remains about the same, the two components have changed proportionately to one another. Since the growth curves indicate a gain in body weight of all animals during the two-week period, we must conclude that those groups which show no general upward or downward deflection of the ratios have had a mineral ash increase proportional to the gain in weight. Such is the case in all the tissues studied of the ad libitum-fed animals.

The recovering phosphorus-deficient animals, however, reveal a different pattern for femurs than for teeth. The body weight of these animals during the two-week period rises faster than the mineral content of their teeth but slower than the mineral content of their femurs. The fact that the ratio of body weight to ash weight of the teeth is lowest during the first few days of recovery suggests that the bulk of the mineral increase in teeth occurs during this early interval. In time, the acquisition of inorganic salts declines and the ratio of body weight to ash increases. The bones, on the other hand, acquire the greater portion of their mineral salts during the later part of the two-week period. The response of bones to recovery from phosphorus deficiency thus takes place over a more protracted period than does that of the teeth.

An estimate of the amount of mineral salts which actually can enter the uncalcified dentin and bone matrix was made as follows: Incisors, molars, and femurs from phosphorus-deficient animals were prepared. A tracing was made of each of the sections with the aid of a camera lucida. The uncalcified dentinoid and osteoid, characterized by their eosinophilic staining, were outlined in the drawing along with the entire section. The tracing was then cut apart to separate the portions representing the uncalcified areas from the remainder of the specimen. The two fractions of paper were weighed, and the resulting values were averaged and expressed as per cent of uncalcified matrix in the total organic material. It is obvious that this method compares these two portions of matrix on a volume basis since the tracing paper allows no differences for density of the tissues. It was determined that the amount of uncalcified matrix in both incisors and molars is about 8 per cent by volume. Data (not presented) obtained on the amount of organic matrix in a whole tooth revealed that it comprises about 25 per cent of the wet weight. The amount of uncalcified matrix must represent a fraction of this.

Eight per cent of the total organic component of the tooth is equal to 2 per cent of the wet weight of the tooth. If this 2 per cent were calcified to its

maximum degree, its weight would increase by two to three times since the ash:matrix ratio of teeth falls in that range. A total change of no more than 6 per cent of the wet weight of a tooth could therefore be expected if complete calcification of the dentinoid seams occurred. Such a change might easily be concealed by the wide fluctuations in ash content found in the experimental animals. Phosphorus-deficient animals' long bones, by way of comparison, have almost 50 per cent of their organic material, exclusive of marrow, as uncalcified osteoid or cartilage. This aids in visualizing why the bone mineral alterations which accompany phosphorus deficiency are so much greater than those of the teeth.

The data on the pair-fed animals are more complex than those of the other two groups. When teeth are analyzed, the pair-fed animals resemble the phosphorus-deficient ones. With the femurs as the basis of comparison, the pair-fed animals respond more nearly like the ad libitum-fed controls. Since the experimental period began as a deficiency of phosphorus, the degree of recovery can be measured by the proximity of a structure to normal. The femurs of the pair-fed animals have responded to such degree that at the end of two weeks they resemble the normal or ad libitum controls. The teeth have lagged behind and still retain some characteristics of the phosphorus-deficient ones.

As to the histologic evidence of calcific aberrations in dentin of rats which are phosphorus deficient, it seems important to point out that some of the studies[1] used animals raised on the Steenbock-Black diet. This ration, although of historical interest, is now known to be deficient in many components. It is also worthy of note that the degree of deficiency is an important factor in the final outcome of a study. The use of a purified diet extremely low in phosphorus by Coleman, Becks, Copp, and Frandsen[3] and by Copp, Chace, and Duffy,[4] has resulted in a degree of deprivation not encountered in this study.

The weight gain which accompanies recovery from phosphorus deficiency due to the restitution of phosphorus is of interest. Vitamin D-stimulated recovery from this condition in chick and rats is associated with a loss of body weight.[18] The discrepancy is probably the result of the ample supply of phosphorus given our animals. Vitamin D presumably acts to mobilize phosphorus for the skeleton.[4, 16, 17] The feeding of phosphorus accomplishes the same thing without the necessity of depleting the soft tissues for this purpose.

SUMMARY AND CONCLUSIONS

1. In rats recovering from phosphorus deficiency by addition of phosphorus to their diets, the mineral ash content of the teeth increases more slowly than the gain in body weight.

2. The femurs of these animals acquire mineral ash faster than the body weight gain.

3. The teeth of the pair-fed animals respond in the same way as those of the phosphorus-deficient ones.

4. The pair-fed animals' femurs react similarly to those of the ad libitum-fed controls.

5. The discrepancy between histologic and chemical findings in teeth of phosphorus-deficient rats may be explained in part by comparing restricted areas capable of response in a tooth to the over-all weight of the structure.

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STUDIES ON THE EFFECT OF SELECTIVE DESALIVATION ON THE DENTAL CARIES INCIDENCE OF ALBINO RATS

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INCREASED rates of initiation and progression of carious lesions after surgical removal of some or all of the principal salivary glands have been reported in the laboratory rat under a variety of conditions.[1-5] Similarly, a substantial increase in susceptibility to tooth decay has been observed in Syrian hamsters and in cotton rats after the extirpation of the major salivary glands.[6-8]

Cheyne[2] conducted an experiment to determine which of the salivary glands contributed most to the maintenance of the teeth while the rats were being fed the Hoppert-Webber-Canniff coarse particle, caries-producing diet.[9] The greatest severity of caries was observed in the group where the parotid and submaxillary glands had been extirpated. The present studies were undertaken to determine in greater detail to what extent relative degrees of caries could be obtained by extirpation of single pairs or combinations of pairs of salivary glands.

EXPERIMENTAL

Albino rats of the Sprague-Dawley strain were used in these studies. At the beginning of each of the two experiments, the animals were approximately 21 days old and weighed 35 to 50 grams. The rats were placed in individual wire cages with screen bottoms and fed the purified caries-producing ration 100 ad libitum throughout the entire experimental periods.[10] In Experiment 1, the animals were divided into eleven groups consisting of eight rats each. After one week to permit adaptation to the purified ration, single pairs of salivary glands or combinations of pairs of glands were extirpated under ether anesthesia according to the schedule shown in Table I. No attempts were made to remove the minor sublingual glands which lie at the base of the tongue. At the end of fifteen weeks the animals were sacrificed by ether.

To confirm the results obtained in Experiment 1, and to try to obtain information as to why the removal of combinations of glands had not produced greater increases in the caries scores than had been caused by the removal of single pairs of glands, an additional 120 white rats were obtained from the same source and were divided into ten groups of twelve rats each. The operations were performed according to the schedule in Table II. Since there was no reason to believe that the caries scores of another group of intact rats which had been sham-operated would differ appreciably from the low caries

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incidence of the sham-operated group in Experiment 1, no sham-operated group was included in Experiment 2. The weight of each pair of glands removed at the time of operation in this experiment was recorded to obtain baseline information on the ratio of gland weight to body weight. At the end of eleven weeks, Experiment 2 was terminated by sacrificing the animals with ether.

TABLE I

DENTAL CARIES EVALUATIONS FOR EXPERIMENT 1

GROUP	NO. OF ANIMALS	GLANDS REMOVED	AVERAGE NUMBER OF CARIOUS TEETH		AVERAGE NUMBER OF CARIOUS LESIONS		AVERAGE EXTENT OF CARIOUS LESIONS	
			AVG.	S.E.M.*	AVG.	S.E.M.*	AVG.	S.E.M.*
1	8	Control	0.6	0.3	0.8	0.4	0.9+	0.5+
2	8	Sham control	0.6	0.5	0.8	0.5	1.3+	0.9+
3	8	Extraorbital lacrimals	1.8	0.8	1.8	0.8	2.6+	1.1+
4	7	All on left side						
		Total	4.0	0.7	6.3	1.2	15.4+	4.2+
		R. side	0.7	0.4	0.7	0.4	1.0+	0.6+
		L. side	3.3	0.5	5.6	1.0	14.4+	3.4+
5	8	Complete desalivation	10.4	0.8	23.8	3.3	93.0+	19.1+
6	7	Parotids	8.4	0.9	12.7	2.1	34.9+	8.1+
7	8	Sublinguals	1.5	0.7	1.5	0.7	2.8+	1.5+
8	8	Submaxillaries	4.4	0.8	5.6	1.1	12.7+	3.5+
9	6	Parotids and submaxillaries	9.7	1.8	16.7	2.8	52.3+	11.6+
10	6	Sublinguals and submaxillaries	5.3	1.6	9.2	2.9	21.5+	8.5+
11	7	Parotids and sublinguals	5.1	1.4	8.4	2.9	19.6+	1.8+

*Standard error of the mean.

TABLE II

DENTAL CARIES EVALUATIONS FOR EXPERIMENT 2

GROUP	NO. OF ANIMALS	GLANDS REMOVED	AVERAGE NUMBER OF CARIOUS TEETH		AVERAGE NUMBER OF CARIOUS LESIONS		AVERAGE EXTENT OF CARIOUS LESIONS	
			AVG.	S.E.M.	AVG.	S.E.M.	AVG.	S.E.M.
1	12	Control	1.6	0.5	1.9	0.7	4.8+	1.8+
2	11	Extraorbital lacrimals	1.4	0.4	1.5	0.4	3.5+	1.1+
3	11	All on left side						
		Total	5.2	0.7	7.6	1.0	18.8+	2.8+
		R. side	1.7	0.4	2.2	0.5	5.3+	1.2+
		L. side	3.5	0.5	5.5	0.8	14.5+	2.2+
4	10	Complete desalivation	11.6	0.3	25.7	1.3	89.8+	7.3+
5	11	Parotids	6.6	1.0	10.2	1.7	25.6+	5.4+
6	11	Sublinguals	3.9	0.9	5.1	1.1	10.5+	2.6+
7	9	Submaxillaries	5.9	0.9	9.1	1.8	23.9+	4.8+
8	11	Parotids and submaxillaries	6.8	0.9	11.8	2.5	35.5+	12.3+
9	11	Sublinguals and submaxillaries	5.4	1.2	9.2	2.5	21.5+	7.2+
10	10	Parotids and sublinguals	6.6	1.3	11.6	2.7	34.4+	8.9+

Throughout both experiments the body weights of each rat were recorded bimonthly. In both studies at the time of sacrifice, each pair of remaining

glands was removed and the weight recorded for the determination of ratios of gland weight to body weight of the experimental animals compared to the control rats.

After sacrifice, the head of each rat was removed and placed in 95 per cent ethanol. After fixation for forty-eight hours, the heads were cleaned of muscle and soft tissue, and the jaws separated to facilitate the evaluation of the number and size of carious lesions by the method used for the cotton rat.[11]

RESULTS

Carious lesions in the albino rat were found almost without exception to occur deep in the occlusal sulci between the cusps of the molars as described elsewhere.[10] In the two experiments reported herein, the type and location of the carious lesions were the same as in other investigations regardless of the gland or the number of glands removed.

The average number of carious teeth, the average number of carious lesions, the average extent of carious lesions, and the standard errors of the means for each of these three criteria for the rats in Experiments 1 and 2 are presented in Tables I and II, respectively. The critical ratios of the difference between averages to the standard error of the difference between means for both studies are shown in Table III.

The control group in both experiments had very low rates of caries incidence which were typical of the fairly high resistance of the particular strain of rats with which we were dealing. The sham-operated control group in Experiment 1 had a similarly low incidence and extent of carious lesions. This indicated that the operative procedure, per se, and the several-day recovery period were not responsible for any part of the increase in caries incidence where one or more pairs of glands were removed.

The removal of the extraorbital lacrimal glands or the sublingual glands produced no significant change in the incidence of caries as judged by the three criteria used in its estimation. However, the elimination of the submaxillaries, or the parotids, or complete desalivation resulted in moderately severe to very severe decay. Highly significant increases in the average number of carious teeth, the average number of carious lesions, and the average extent of carious lesions for these operated animals over the values obtained for the controls occurred in each of these experimental groups. The removal of the parotids in each experiment caused numerically greater caries incidences than the removal of the submaxillaries. In Experiment 1, this difference was statistically highly significant, but the difference was not significant in Experiment 2. When the data from both experiments were pooled, the increase in caries incidence after parotid removal was found to be significantly greater than after the removal of the submaxillaries.

Similar highly significant increases over the control group were observed in the caries scores for the group where both the parotid and submaxillary glands were removed. The values for the caries incidence for the submaxillary-sublingual and the parotid-sublingual groups were significantly increased

compared to control animals. In the latter two groups, some of the differences were significant at the 5 per cent level rather than at the 1 per cent level of significance observed uniformly for all previous groups.

TABLE III

STATISTICAL COMPARISON* OF DENTAL CARIES EVALUATIONS IN EXPERIMENTS 1 AND 2

	EXPERIMENT 1			EXPERIMENT 2		
COMPARISON OF GROUPS	AVERAGE NO. OF CARIOUS TEETH	AVERAGE NO. OF CARIOUS LESIONS	AVERAGE EXTENT OF CARIOUS LESIONS	AVERAGE NO. OF CARIOUS LESIONS	AVERAGE NO. OF CARIOUS LESIONS	AVERAGE EXTENT OF CARIOUS LESIONS
Control vs. sham control	0.0	0.0	0.4			
Control vs. extraorbital lacrimals	1.4	1.1	1.5	0.4	0.6	0.7
Control vs. desalivated on left side	4.4	4.6	3.4	4.4	4.2	4.2
Control vs. complete desalivation	14.8	6.9	4.8	15.3	16.7	11.3
Control va. parotids	8.0	5.5	4.2	4.6	4.6	3.8
Control vs. sublinguals	1.1	0.9	1.2	2.3	1.8	1.8
Control vs. submaxillaries	4.4	4.6	3.4	4.0	3.8	3.7
Control vs. parotids-submaxillaries	7.5	5.7	4.4	5.2	3.8	2.5
Control vs. submaxillaries-sublinguals	3.0	2.9	2.4	3.0	2.8	2.3
Control vs. parotids-sublinguals	3.2	2.6	1.8	3.6	3.5	3.2
Desalivated on left side-left side vs. right side	4.2	4.5	3.9	2.8	3.5	3.7
Right side of rats desalivated on left side vs. one side of controls	1.6	1.1	0.8	0.9	1.7	2.2
Complete desalivation vs. desalivated on left side only	6.1	5.0	3.9	26.7	11.4	8.9
Complete desalivation vs. parotids	1.5	4.8	2.8	4.2	7.0	6.9
Complete desalivation vs. sublinguals	8.1	6.4	4.7	8.1	12.1	10.2
Complete desalivation vs. submaxillaries	5.0	5.2	4.1	4.7	7.5	7.5
Complete desalivation vs. sublinguals-submaxillaries	2.8	3.3	3.4	5.2	5.8	6.7
Complete desalivation vs. submaxillaries-parotids	0.5	1.6	1.8	5.3	4.9	3.8
Complete desalivation vs. sublinguals-parotids	2.9	3.5	3.4	3.8	4.7	4.8
Parotids vs. submaxillary-parotids	0.9	1.2	1.2	0.2	0.5	0.7
Parotids vs. sublinguals-parotids	1.9	1.2	1.2	0.0	0.3	0.9
Parotids vs. submaxillaries	3.3	2.9	2.5	0.5	0.5	0.4

*Figures given are critical ratios. The critical ratio is the ratio of the difference between two means to the standard error of the difference between the means. Wherever the critical ratio is less than 2.0, the difference between the means is considered to be statistically insignificant; when the critical ratio is between 2.0 and 2.9, the difference is of borderline significance; when the critical ratio is 3.0 or higher, the difference is highly significant.

In the groups of rats which were desalivated on the left side only, the greater incidence of carious lesions on the operated side than on the intact side represented a highly significant increase. Likewise, the over-all increase in caries in the rats operated on one side as compared to the controls was highly significant. However, the incidence of carious lesions on the intact side was not appreciably higher than on either side of the unoperated controls or the sham controls. In addition, the total caries incidence in rats which were desalivated on one side only was highly significantly less than in the completely desalivated rats.

In every case, the rats which had had all of the major salivary glands removed had a greater average number of carious molars, a greater average number of carious lesions, and a greater average extent of carious lesions than the rats which had single pairs or combinations of pairs of glands removed. In the case of the two groups of rats in each experiment which had had only the extraorbital lacrimal glands, or only the sublingual glands removed, the greater caries incidence in the completely desalivated rats was of very high significance. Where only the submaxillaries or the parotids were removed, the larger caries incidence in the completely desalivated rats was highly significant, but at a somewhat lower level than for the previous group. In one comparison, the increase was statistically insignificant; the average number of carious molars of the completely desalivated group in Experiment 1 was 10.4, which did not constitute a significantly greater value than the 8.4 average for the group with only the parotids removed. The latter incidence of carious molars constitutes an unusually and unexplainably high attack rate for the molars, in view of the accompanying low number of carious lesions and low extent, each of which represented significantly lower values than those of the rats which were completely desalivated.

The completely desalivated rats had a highly significantly greater caries incidence than the rats which had both the sublinguals and the submaxillaries removed and than the rats which had both the sublinguals and parotids removed. A discrepancy between the two experiments was noted in the difference between the completely desalivated rats and those in which both the submaxillary and parotid glands had been removed. In Experiment 1, the caries incidence with respect to all three criteria of estimation was numerically appreciably higher in the completely desalivated rats than for the submaxillary-parotid group, but these differences were not sufficiently great to be statistically significant. In Experiment 2, the difference between these groups was highly significant. The higher standard error of the mean for the completely desalivated group in Experiment 1 than in Experiment 2 may account for this lack of significance.

The groups of rats which had either both the sublingual and parotid glands removed or both the submaxillary and parotid glands removed did not have significantly greater caries incidences than the rats which had only the parotids removed.

The average body weights, the average absolute gland weights, and the average ratios of gland weight to body weight are recorded in Table IV. The ratios of gland weight to body weight are enclosed in parentheses. A statistical comparison of absolute gland weights was not performed; examination of these data in Table IV reveals moderate to large increases in the average absolute weight of the remaining major salivary glands when either the parotids, sublinguals, submaxillaries, parotids and submaxillaries, sublinguals and submaxillaries, or parotids and sublinguals were removed. The results indicate that under these experimental conditions a compensatory increase in size of the remaining major salivary glands does occur. Even when the small

TABLE IV

BODY WEIGHTS, ABSOLUTE GLAND WEIGHTS, AND RATIOS OF GLAND WEIGHT TO BODY WEIGHT

GLANDS REMOVED	AVERAGE BODY WEIGHT (GM.)		AVERAGE WEIGHT OF EXTRAORBITAL LACRIMALS (MG.)		AVERAGE WEIGHT OF SUBLINGUALS (MG.)		AVERAGE WEIGHT OF SUBMAXILLARIES (MG.)		AVERAGE WEIGHT OF PAROTIDS (MG.)	
	EXP. 1	EXP. 2	EXP. 1	EXP. 2	EXP. 1	EXP. 2	EXP. 1	EXP. 2	EXP. 1	EXP. 2
Control	220.6	220.7	193.5 (89.3)	191.1 (87.1)	66.8 (30.6)	71.2 (32.2)	340.2 (155.1)	311.9 (142.1)	215.0 (97.5)	273.0 (125.0)
Sham control	223.5	–	183.2 (83.3)	–	63.6 (29.1)	–	362.5 (165.8)	–	263.5 (108.5)	–
Extraorbital lacrimals	229.6	237.7	–	–	78.5 (36.6)	71.2 (30.1)	336.0 (155.3)	376.2 (160.4)	224.0 (102.9)	306.3 (129.9)
All on left side	200.6	219.2	92.1 (45.9)	113.2 (52.0)	39.6 (19.6)	46.4 (21.8)	207.7 (103.7)	215.7 (100.3)	153.0 (75.8)	175.2 (80.3)
Complete desalivation	185.1	198.2	–	–	–	–	–	–	–	–
Parotids	215.7	227.8	190.3 (88.1)	189.1 (83.6)	77.7 (36.4)	78.8 (34.8)	404.2 (186.9)	366.6 (158.8)	–	–
Sublinguals	215.7	224.3	204.0 (95.4)	213.0 (95.0)	–	–	357.7 (168.1)	346.7 (153.1)	272.3 (132.0)	376.2 (168.3)
Submaxillaries	217.5	211.8	189.6 (88.3)	196.5 (93.3)	111.5 (52.4)	106.0 (50.7)	–	–	276.8 (121.4)	393.8 (186.1)
Parotids and submaxillaries	219.0	195.9	220.0 (101.1)	210.3 (115.9)	135.0 (70.3)	124.8 (61.3)	–	–	–	–
Sublinguals and submaxillaries	206.5	195.9	186.6 (90.5)	202.9 (104.1)	–	–	–	–	346.5 (165.2)	416.8 (217.0)
Parotids and sublinguals	200.5	217.5	207.9 (103.5)	215.3 (99.5)	–	–	411.4 (201.9)	401.5 (185.2)	–	–

Values in parentheses are for ratio of gland weight to body weight.

major sublingual glands alone were extirpated, the parotid glands increased in size. Evidently the extraorbital lacrimal glands became appreciably enlarged only when both the parotid and submaxillary glands were absent.

In Table V is presented the statistical comparison of the ratios of gland weight to body weight in both the control group and the operated groups. It is noteworthy that in the groups where the parotid glands were extirpated, considerable enlargement of the intact submaxillary glands took place in both studies, although more markedly in Experiment 1. A similar increase in the ratio of parotid weight to body weight occurred when the sublinguals were removed, of the sublinguals and the parotids when the submaxillaries were removed, of the sublinguals when both the submaxillaries and parotids were taken out, of the parotids when the sublinguals and submaxillaries were absent, and finally, of the submaxillaries when the parotids and sublinguals were extirpated. The increase in ratio of the weight of the remaining glands to body weight in each instance is significant in comparison to the controls.

TABLE V

STATISTICAL EVALUATION OF THE INCREASES IN THE SALIVARY GLAND-BODY WEIGHT RATIOS OBSERVED IN EXPERIMENTS 1 AND 2

	CRITICAL RATIOS OF THE GREATER WEIGHT OF THE REMAINING SALIVARY GLANDS AS COMPARED TO THE WEIGHTS OF THE SAME GLANDS IN INTACT CONTROLS							
	EXTRAORBITAL LACRIMALS		SUBLINGUALS		SUBMAXILLARIES		PAROTIDS	
GLANDS REMOVED	EXP. 1	EXP. 2	EXP. 1	EXP. 2	EXP. 1	EXP. 2	EXP. 1	EXP. 2
Extraorbital lacrimals	--	--	1.7	0.5	1.0	1.9	0.8	0.5
Parotids	0.2	0.7	3.1	2.0	3.0	3.3	--	--
Sublinguals	0.7	1.2	--	--	1.0	1.4	2.8	4.6
Submaxillaries	0.1	1.0	4.7	3.9	--	--	3.4	6.2
Parotids and submaxillaries	2.4	4.9	7.5	7.5	--	--	--	--
Sublinguals and submaxillaries	0.1	2.5	--	--	--	--	5.9	6.5
Parotids and sublinguals	0.5	1.8	--	--	3.3	7.4	--	--

DISCUSSION

The white rat becomes very susceptible to dental decay when the major salivary glands are extirpated. This is particularly evident when the animal is maintained on a caries-producing diet containing 67 per cent sucrose. Invariably under these conditions severe carious breakdown of the molar teeth of the strain of rats used in these studies is observed in twelve to fifteen weeks. In other studies in these laboratories, it has been shown that even strains of rats which are genetically highly caries-resistant and which have been maintained on natural diets until long after tooth development has been completed will develop a relatively high incidence of carious lesions after desalivation.

In the experiments here reported, relative degrees of tooth decay were produced by variations in the number and type of gland extirpated. The loss of the extraorbital lacrimal and major sublingual glands was of minor importance in caries production in the white rat. The white rat appeared to be

amply supplied with salivary secretions from the intact submaxillary and parotid glands. However, the loss of either the submaxillary or parotid glands resulted in a serious inability of the remaining glands to maintain caries resistance, and severe decay ensued, although never as extensive as that which occurred in completely desalivated animals. The results point to the singular importance of the parotid glands for the resistance to dental decay in the white rat followed closely by the submaxillary glands, with the major sublinguals and the extraorbital lacrimal glands being of an entirely different order of importance.

It was anticipated that, when combinations of the submaxillary and sublingual, parotid and sublingual, and parotid and submaxillary glands were removed, larger caries scores would be obtained than those observed when single pairs of these glands were removed. However, such was not the case; instead those groups with pairs of glands removed had dental caries incidences approximating those caused by the removal of the most important single gland of the combination. Possibly this unexpected finding can be attributed entirely or in part to the enlargement of the remaining glands when these combinations of pairs of glands were extirpated. Such enlargement occurred even when single pairs of glands were extirpated. Unfortunately no observations were possible to determine if the increase in the size of the gland was accompanied by an increase in salivary flow or in a change in the composition of the saliva produced.

The comparison of the caries scores of the completely desalivated animals and of the various operated groups again revealed almost uniformly significant differences. Yet these differences were on a generally graded level of significance, with the magnitude of the difference related to the severity of gland deprivation.

The data collected in Experiment 2 remarkably reproduce the results obtained in Experiment 1, with the exceptions which have been commented upon in the section on Results. The one case was the comparison between groups when only the parotids and only the submaxillaries had been removed; the other exception was in the comparison of completely desalivated rats with those that had both the submaxillaries and parotids removed. In both cases the numerical trend was in the same direction for the two experiments, but in one experiment the differences between the results were insignificant whereas in the other experiment the results were highly significant. In both of these exceptions, when the results of the two experiments were pooled, the differences were found to be statistically highly significant. Since the methods used in both studies were exactly the same, it is difficult to explain these differences in the statistical interpretation of the observations. Possibly some unrecognized respiratory infection or failure to randomize the selection of groups or other unexpected variable precipitated these differences. In any case, the points in which differences occur are of insufficient importance to obscure the interpretation of the major portion of the data.

CONCLUSIONS

Relative amounts of dental decay were produced in the white rat by removal of one or more pairs of salivary glands.

Sham operations did not increase the caries incidence above that of the control rats. The removal of only the extraorbital lacrimal glands or only the major sublingual glands resulted in no significant increases in caries incidence as compared to controls. Highly significant increases in caries resulted in those animals which were deprived of either the submaxillary or parotid glands.

Extirpation of the major salivary glands on one side resulted in caries scores on that side which were significantly greater than on the intact side, and did not cause a significant increase in the caries scores on the intact side.

In groups of rats where combinations of the submaxillaries and sublinguals, parotids and submaxillaries, or parotids and sublinguals were extirpated, the increase in caries incidence varied from barely significant to highly significant in comparison with controls. These scores were not uniformly greater than when only one of the two pairs was removed, possibly due in part, to the increase in weight which was observed in the remaining glands.

Removal of the extraorbitals, the sublinguals, the submaxillaries, the parotids, or combinations of pairs of these glands resulted in significantly less decay than when all these major salivary glands were removed simultaneously.

These studies demonstrated that the parotids were the most important of the salivary glands in relation to caries resistance in the white rat. The submaxillaries exerted almost equal influence, while the major sublinguals and extraorbital lacrimal glands were of much lesser importance in this respect.

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FREE AMINO ACIDS OF HUMAN SALIVA; A CHROMATOGRAPHIC INVESTIGATION

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INTRODUCTION

UNTIL recently, comparatively little was known of the quantity of free amino acids present in human saliva. This could be attributed to the lack of a good and reliable analytic procedure for the determination of the amino acid content of body fluids. It appeared, moreover, that the amount of free amino acids present in saliva was extremely small.[1] Later, using microbiologic methods of analysis, Kirch, Kesel, O'Donnell, and Wach[2] determined the content of different amino acids in the stimulated saliva of eighteen individuals. They proved the presence of sixteen amino acids in saliva. The investigations of Goldberg, Gilda, and Tishkoff,[3] in general, agreed qualitatively with these results; these authors demonstrated thirteen amino acids in saliva by means of paper partition chromatography. Rosebeek[4] found fourteen amino acids by circular paper chromatography.

The microbiologic method for the determination of different amino acids in a mixture is unsuitable, in general, for an extensive investigation, as it is impossible by this method to determine all the amino acids present, and the specificity of the analyses is not always of the same degree.

Paper chromatographic methods are less suitable for quantitative purposes. Stein and Moore[5, 6] described an excellent method for the separation and quantitative determination of amino acids, and pointed out many practical applications of it. This method, working chromatographically, with the aid of an ion exchanger, was used by us in the quantitative determination of free amino acids in human saliva.

EXPERIMENTAL

The technic of the chromatographic analysis requires rather intricate apparatus for the collection of the various fractions.[8]

For the fractionation of mixtures of amino acids by elution analysis, columns of Dowex-50, a sulphonated polystyrene resin, are used. The separation of the acid and neutral amino acids is carried out on a 0.9 by 100 cm. column with the aid of buffers at pH 2.5, 2.9, 3.4, 4.25, and 6.7, the working temperatures being 37°, 50°, 75°, and 25° C. The basic amino acids are determined by means of a short column (0.9 by 15 cm.) by elution with buffers pH 5.0, 6.8, and 6.5 (temperature 25° C.). The determination of the content of the various fractions is based on a photometric determination of the amino acids. The

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underlying principle is a reaction of the amino acid with ninhydrin, in which diketohydrindylidene-diketohydrindamine is formed as a blue condensation product, with an absorption maximum at 570 mμ.

The Huisman apparatus,[9] based on the time principle, was used as fraction collector. This simplified arrangement also proved to be highly suitable for the determination of salivary amino acids. Pertinent literature[5-8] presents a complete discussion of theory and practice of this method of amino acid determination.

The saliva is collected in a beaker. Each person stimulates the flow of about 110 to 120 ml. of saliva by chewing paraffin. The necessary deproteinization is carried out directly following this saliva production, by means of ultrafiltration[10,12] through a cellophane filter at a temperature of 0° C. and a pressure of 200 mm. Hg.

Of the crystal-clear filtrate obtained in this manner, 100 ml. is evaporated to dryness in vacuo in a rotating Craig[11] apparatus. The residue is dissolved in 10 ml. water and (because the pH of an amino acid mixture which is to be separated chromatographically into its components must be between 2.4 and 2.6) is brought to the desired pH with concentrated HCl. Four milliliters of the solution thus obtained, corresponding with 40 ml. of the original saliva, is chromatographed on the column. This quantity usually proves quite sufficient for a chromatogram. The analysis is performed in one-half of the quantity of the eluted fractions; the curve of a chromatogram thus obtained shows a figure corresponding with one-half the quantities of the amino acids present.[12]

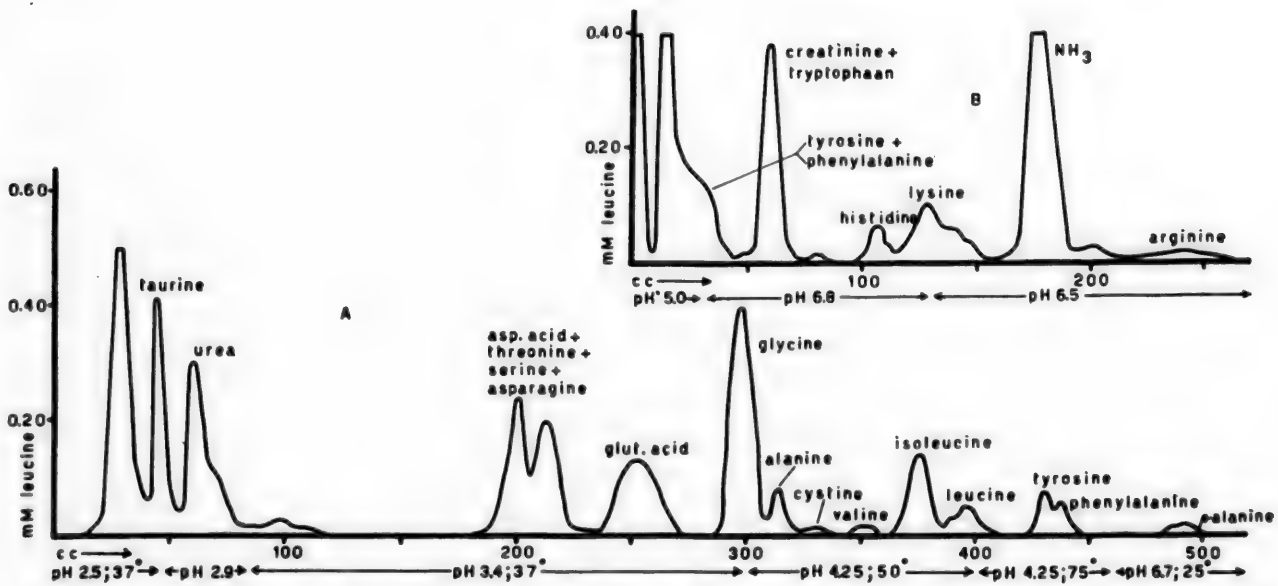
RESULTS AND DISCUSSION

The chromatograms reproduced here show the result of the separation of the amino acids in saliva ultrafiltrate originating from five test persons. Figs. 1 to 5 show these effluent concentration curves, the elution of the amino acids being indicated by buffers[6] of increasing pH; the amino acid concentration in each tube is expressed in leucine equivalents.[5] Figs. 1, *A* to 5, *A* show curves in which the separation of the amino acids is obtained by means of a 0.9 by 100 cm. Dowex-50 column. In Figs. 1, *B* to 5, *B* the separation of the basic amino acids is brought about with a 0.9 by 15 cm. column.

The content of the various salivary amino acids found in this way is given in Table I. This shows that aspartic acid and valine (and possibly β-alanine) are present in only very low concentrations. The small amount of arginine found is a remarkable feature, in contrast to the observations of other authors.[2] The presence of cystine and methionine was demonstrated only in an occasional case. It is possible that failure to find these amino acids can be explained because they occur only in minute concentrations. The insensitive reaction of cystine with ninhydrin may perhaps be the reason that this amino acid is absent.

The determination of the content of the basic amino acids on the short 0.9 by 15 cm. column is made somewhat difficult because at the same time a number

Fig. 1.



Figs. 1-5.—Separation of the ninhydrin-positive components from ultrafiltrates of five human saliva samples. In all cases a quantity corresponding with 40 ml. of the original saliva was chromatographed, except case 1, *B*, 4, *A* and 5, *A*, where quantities, corresponding, respectively, to 20, 30, and 30 ml. were chromatographed. *A*, Chromatograms of the 0.9 by 100 cm. column of Dowex-50. *B*, Chromatograms of the 0.9 by 15 cm. column of Dowex-50.

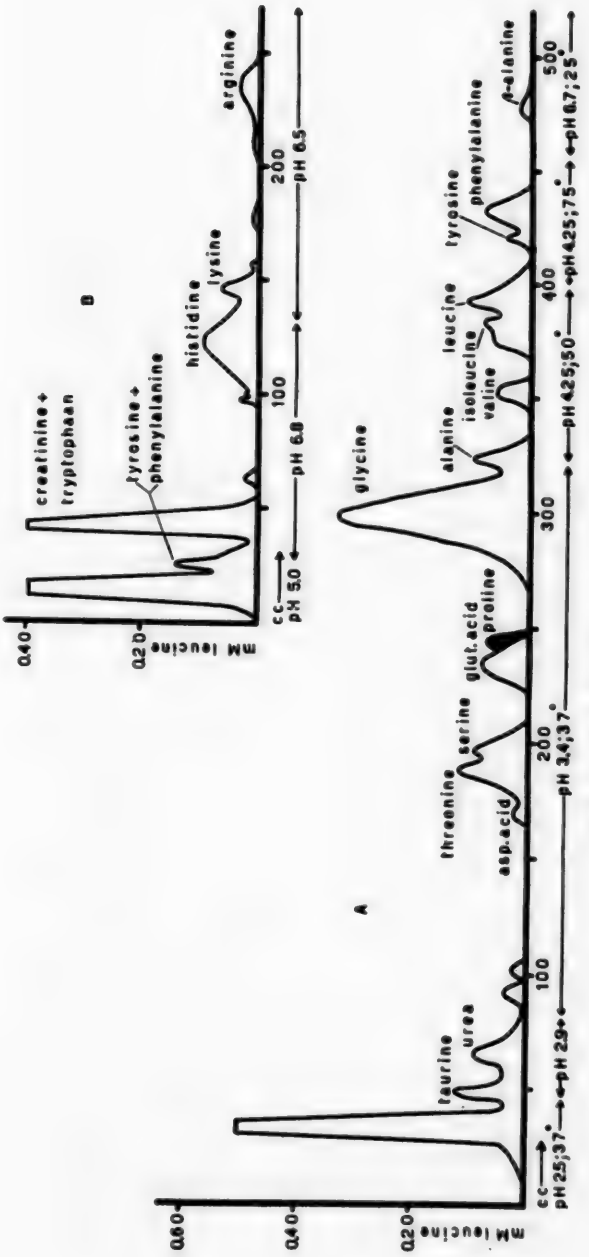


Fig. 2. *(For legend see opposite page.)*

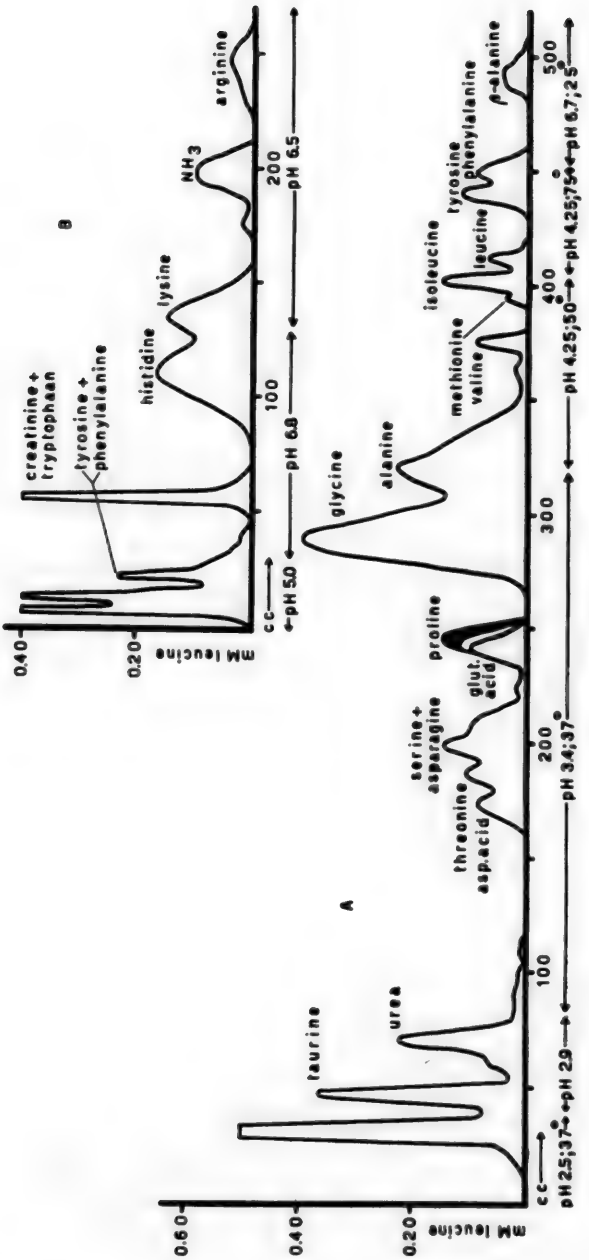


Fig. 3. (*For legend see page 250.*)

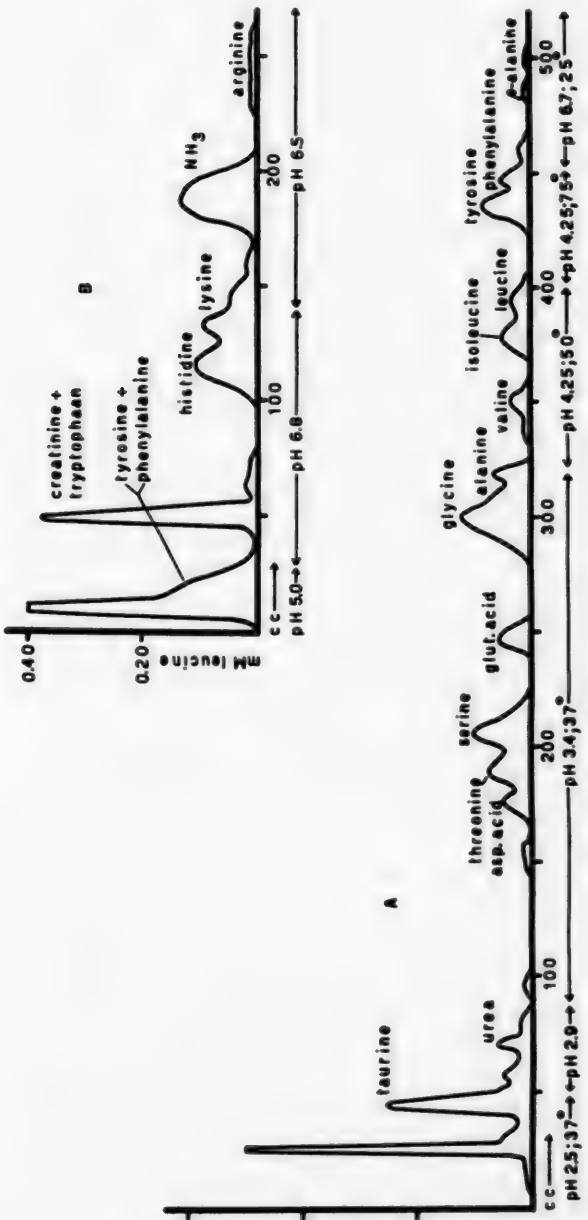


FIG. 4. (*For legend see page 250.*)

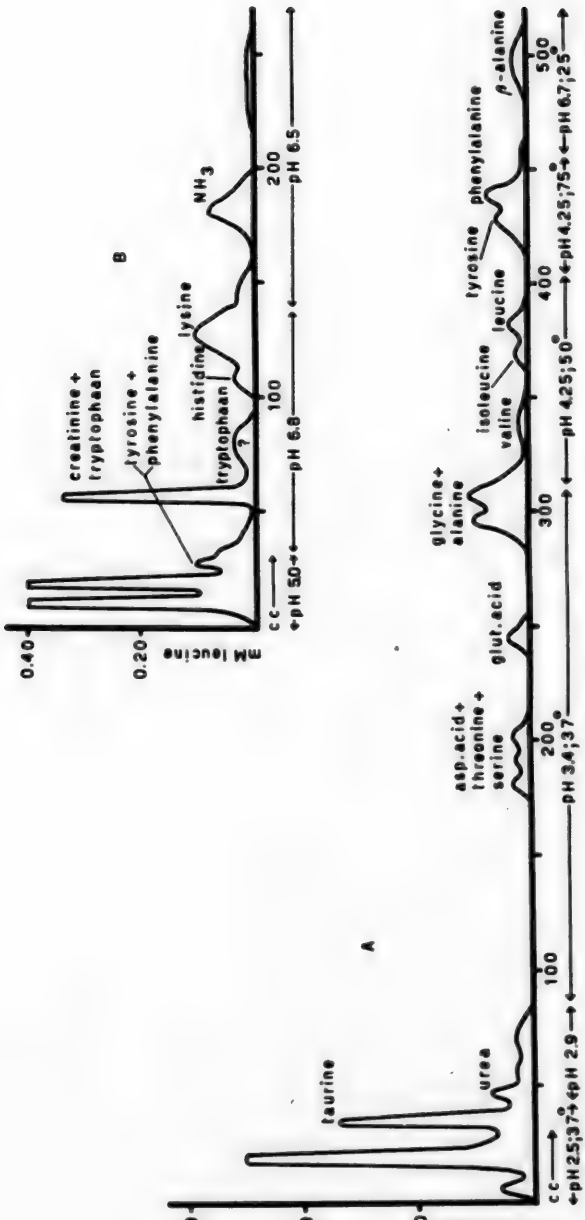


Fig. 5. (*For legend see page 250.*)

of peaks are obtained originating from unknown ninhydrin-positive substances, while, moreover various components overlap one another. Stein[7] mentioned a coincidence of lysine with methylhistidine and other components, and of β-alanine with α-aminobutyric acid. Thus the determination of the tryptophan content by this method is made doubtful by an overlapping with adjoining peaks (creatinine and histidine). Tryptophan may be present in an occasional case only.

TABLE I

AMINO ACID CONTENT (IN MILLIGRAMS PER CENT) IN ULTRAFILTRATE OF HUMAN SALIVA

AMINO ACID	SPECIMEN				
	I	II	III	IV	V
Taurine	0.67	0.20	0.56	0.45	0.59
Aspartic acid	0.49	0.05	0.27	0.12	0.08
Threonine		0.32	0.38	0.26	0.07
Serine	0.49	0.31		0.38	0.09
Serine† asparagine	0.54		0.65		
Glutamic acid	0.92	0.58	0.53	0.23	0.16
Proline	—	0.28	0.74	—	—
Glycine	1.56	1.24	1.50	0.49	0.35
Alanine	0.22	0.25	0.91	0.24	0.42
Cystine	0.13	—	—	—	—
Valine	0.09	0.19	0.23	0.12	0.10
Methionine	—	—	0.07	—	—
Isoleucine	0.78	0.47	0.84	0.31	0.14
Leucine	0.32	0.53	0.35	0.28	0.18
Tyrosine	0.43	0.17	0.80	0.58	0.37
Phenylalanine	0.22	0.38	0.49	0.26	0.41
B-alanine†*	0.08	0.05	0.14	0.04	0.06
Tryptophan†	—	—	—	—	0.26
Histidine	0.46	0.94	1.25	0.78	0.27
Lysine†	1.37	0.36	1.21	0.72	0.78
Arginine	0.20	0.24	0.35	0.17	—

*Uncorrected for the presence of other components (α-amino-butyric acid?).
†Uncorrected for possible other components (methylhistidine[7]).

The identity of the peaks can be established by the chromatographic separation of a mixture of known amino acids, because, when the experiment is carried out carefully, the place each component occupies in the chromatogram is constant within narrow limits. The amino acids may also be identified from an increase of the peaks after the addition of known amino acids as tracers to the fluid under analysis.

The values for the salivary amino acids obtained by Kirch, Kesel, O'Donnell, and Wach, from microbiologic investigation, are in most cases higher than the values found by us (Table I). Proline, isoleucine, leucine, tyrosine, histidine, and lysine are in reasonable agreement.

The salivary amino acid contents, determined by the described method, are usually lower than the concentrations of these components in blood plasma (also found chromatographically[12, 13]). In some cases, taurine, aspartic acid, glutamic acid, glycine, isoleucine, and lysine yield values of the order of those of plasma.

Apart from the majority of the known amino acids discovered in this way in saliva, there are also present: taurine, urea, creatinine, and ammonia, and, moreover, a number of unknown substances. The taurine content found by us is also mentioned in Table I; the creatinine and tryptophan contents are difficult to calculate because of overlapping of peaks.

SUMMARY

The ninhydrin-positive components of the saliva of five individuals were fractionated chromatographically by means of Dowex-50 columns. The free amino acids present in human saliva (ultrafiltrate) were quantitatively determined by this method. The presence of at least eighteen amino acids in human saliva has been demonstrated.

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METHOD FOR THE COLLECTION OF SEPARATE SUBMAXILLARY AND SUBLINGUAL SALIVAS IN MAN

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SYSTEMATIC investigation of salivary constituents and of the process of salivary secretion in man requires the collection of separate salivary fractions. Until the present time it has not been possible, with any degree of convenience, to collect the submaxillary and sublingual secretions separately, although collection of parotid saliva by the use of relatively simple devices has been possible for some time.[1, 2] It is the purpose of this report to describe a method for the routine collection of separate submaxillary and sublingual secretions. This method is based on a modification of a device (segregator) used by Pickerill[3] for the collection of mixed submaxillary-sublingual saliva and involves the construction of a separate acrylic appliance for each saliva donor. The acrylic appliance covers an area extending anteroposteriorly from the lingual surfaces of the incisors to the first molar region and buccolingually from the lingual surfaces of the teeth under the tongue to cover the orifices of the submaxillary ducts and the plica sublingualis. The inferior surface of the appliance contains three chambers, each open inferiorly to its full extent. The medial chamber overlies the submaxillary duct orifices; the lateral chambers cover the plica sublingualis. Plastic tubing leads from each chamber through the roof of the appliance to the exterior and serves to conduct the secretion to collection tubes. Each chamber is separated from the other by a rim of acrylic which contacts the tissue of the floor of the mouth and creates an effective seal for each collection chamber. The effectiveness of the seal is indicated by analysis of sodium concentrations of the salivary fractions before and after sodium chloride is injected from the exterior into one chamber. In addition, the marked difference in the physical and chemical character of the secretions indicates that satisfactory separation of the two occurs.

METHOD

The tissue-contacting inferior surface of a typical appliance is illustrated in Fig. 1. The wings of the appliance contain the chambers which cover the plica sublingualis. The insertion of the plastic tube may be seen near the center of each chamber. The body of the appliance contains the chamber for the accumulation of submaxillary saliva and the entrance of the conducting tube.

The following steps are involved in the construction of an appliance: (1) The lingual border of a thin-walled partial denture tray is bent so that the

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plica sublingualis is included within the borders of the tray. The anterior lingual portion of the tray is extended by attachment of base-plate wax to include the orifices of the submaxillary ducts. (2) An alginate impression is made of the lower arch and floor of the mouth. Care is taken to see that the impression includes the area of the floor of the mouth lingually to the sublingual furrow and the submaxillary duct orifices. (3) A stone model is poured. (4) On the

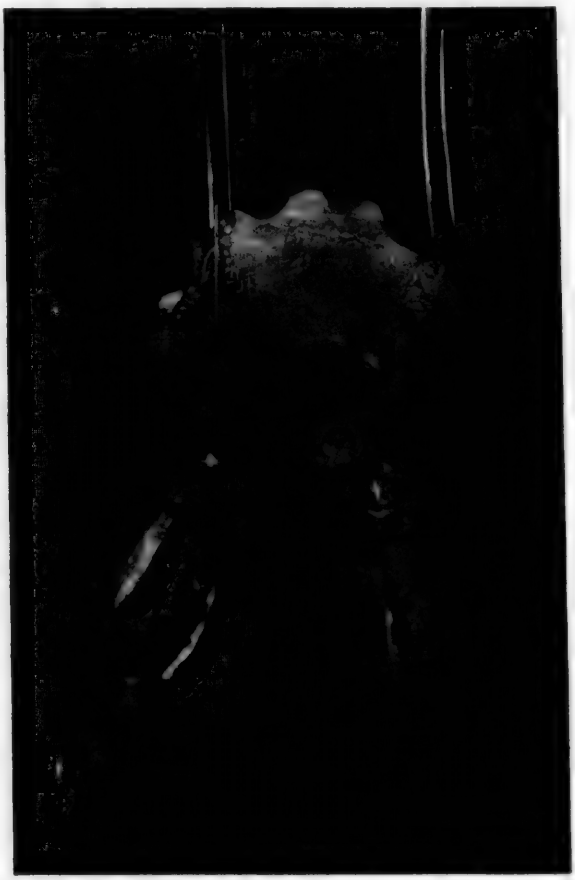

Fig. 1.—Inferior surface of an acrylic appliance used to collect separate submaxillary and sublingual salivas. Note lateral sublingual and medial submaxillary chambers and exit of collection tubes.

model the plica sublingualis and the orifices of the submaxillary ducts are covered with a strip of base-plate wax to a height of several millimeters. The wax is cut away from the region between the plica and the orifices of the submaxillary ducts. This leaves three narrow strips of wax arranged in semicircular fashion on the lingual portion of the model. A six-inch length of one-sixteenth-inch bore plastic tubing is sealed onto the superior surface of each wax strip. (5)

The model is coated with a separating medium, and self-curing acrylic is applied to the model and over the wax strips by sprinkling on the polymer and then wetting this with monomer.[4] This is continued slowly until the acrylic fills the volume between the lingual surfaces of the teeth and the region just lingual to the wax strips. The acrylic is built up to a level sufficient to cover the lingual surfaces of the anterior teeth and the cervical margins of the lingual surfaces of the posterior teeth. The plastic tubes are inclined anteriorly as the acrylic is applied. (6) The acrylic is allowed to polymerize and is then separated from the model. The wax is removed from the three appliance chambers. (7) Slight adjustments are made, if necessary, to permit seating of the appliance. Natural undercuts and adhesion are sufficient to hold the appliance in place in the mouth. (8) The saliva is permitted to accumulate in the chambers and to flow out of the tubes by secretion pressure.

RESULTS AND DISCUSSION

Satisfactory separation of the submaxillary and sublingual salivary fractions by the use of the mandibular appliance or "segregator" requires two conditions: first, an adequate mechanical seal must be obtained between the chambers collecting from the area of the plica sublingualis and the chamber collecting from the orifices of the submaxillary ducts, and second, even prior to collection, mixing of the two secretions must not occur as a result of the spatial relationships of the ducts. Data from chemical analysis of the contents of the chambers indicate clearly that mixing does not occur to any measureable degree after the secretions have reached the appliance from the glands. Radiographic and anatomic evidence is available from the literature to indicate that emptying of sublingual ducts into or beside the submaxillary ducts is a relative rarity and emptying of the submaxillary into a sublingual duct does not occur.

The data presented in Table I demonstrate the efficacy of the mechanical seal between the sublingual and submaxillary chambers. In this experiment, an appliance was prepared which contained two conducting tubes inserted into the right sublingual chamber. After insertion of the appliance, a sample of saliva from each of the chambers was collected. Following this, a small volume of 1 normal NaCl was injected into one of the tubes leading from the right sublingual chamber. Saliva samples were collected from each sublingual and from the submaxillaries as before. The samples were then analyzed for sodium and potassium using a Beckman D U spectrophotometer with flame attachment. As shown in Table I, the sodium concentration of the salivas collected from the left sublingual and from the submaxillary chambers did not increase after addition of sodium chloride to the right sublingual chamber. The sodium concentration of the saliva in the right sublingual chamber rose significantly.

In addition, significant differences in the physical and chemical characteristics of the secretions point to a satisfactory separation of the salivas. The data given in Table II, for example, illustrate the large differences in viscosity of samples from a single donor of parotid, submaxillary, sublingual, and total

TABLE I

ANALYSIS OF FLUID FROM SEGREGATOR CHAMBERS BEFORE AND AFTER ADDITION OF SODIUM CHLORIDE TO THE RIGHT SUBLINGUAL CHAMBER

	BEFORE ADDITION OF NaCl		AFTER ADDITION OF NaCl	
	(MILLIEQUIVALENTS PER LITER)			
	K	Na	K	Na
Submaxillary	20.0	2.0	23.1	2.0
Left sublingual	17.1	2.8	19.5	2.0
Right sublingual	13.8	2.0	13.8	36.8

TABLE II

VISCOSITY IN CENTIPOISES OF STIMULATED* SALIVARY FRACTIONS COLLECTED FROM A SINGLE DONOR

	TOTAL MIXED	PAROTID	SUBMAXILLARY	SUBLINGUAL
Viscosity (centipoises)	2.9	1.5	3.4	13.4

*Saliva stimulated by painting the tongue with 1 per cent acetic acid.

mixed salivas. Viscosity was determined by the use of a modified Ostwald viscosimeter at 30° C. In this case the viscosity of the sublingual saliva was about four times that of the submaxillary and nine times that of the parotid.

The literature concerned with the anatomy of the sublingual glands and the spatial relationship between the submaxillary and sublingual ducts is not entirely consistent. According to Drennan,[5] "The sublingual ducts are about twelve in number. . . ; they leave the superior part of the gland, and . . . open on a series of papillae . . . which are placed along the summit of the sublingual fold. . . . As a rule all the ducts open separately on the summit of the sublingual fold, and apparently none of them join the submandibular duct. Frequently some of those from the anterior and more bulky part of the gland are larger than the others, but the presence of a large duct running alongside the submandibular duct, and opening with or beside it, is very rare in man, although normal in the ox, sheep, and goat." Meschan,[6] from radiographic studies, reported the occasional visualization of a portion of the sublingual gland after injecting the submaxillary duct. In this connection, it is worthy of note that no claim has been made for the emptying of the submaxillary secretion into a sublingual duct. Further, by the use of the segregator it has become apparent that the rate of sublingual secretion is only about 4 per cent of the rate of submaxillary secretion. Consequently, on an anatomic basis, it appears unlikely that contamination of submaxillary saliva by sublingual occurs to any frequent or significant extent, and the reverse possibility, contamination of sublingual saliva by submaxillary, seems extremely remote. The possibility remains of some contamination of both secretions by small buccal glands which may be present in the mucous membrane under the collection chambers. Since, however, the major buccal glands are localized in other regions of the oral cavity, it is unlikely that contamination from this source would be significant.

SUMMARY

The construction and use of a relatively simple appliance for the collection of separate submaxillary and sublingual fractions are described. Chemical and anatomic evidence is presented that separate secretions are, in fact, obtained by the use of this appliance.

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WEATHER CONDITIONS AFFECTING PATIENTS' REACTIONS TO LOCAL ANESTHETICS

II. A Report of 2,708 Consecutive Cases

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EXTENSIVE literature was previously cited[1] on the effect of weather change on patients subjected to drugs,[2, 3, 12] a correlation between weather change and degenerative diseases, such as heart disease and diabetes,[4, 5, 12, 13] weather effects upon allergic manifestations, and weather effects upon infectious diseases.[5-10] Subsequently a study of the effects of weather upon postoperative hemorrhages following tonsillectomies and adenoidectomies was published.[14]

A previous report[1] described in detail the possible relations of local anesthetic reactions to weather data supplied by the United States Department of Commerce Weather Bureau at Los Angeles. In order to establish a broader base from which to draw conclusions, this original study was extended to cover a 5-year period, using the same anesthetic solution* and with no change in any detail.

MATERIALS AND METHODS

This report covers the months from July 1, 1945, through June 30, 1950. All details previously reported[1] were continued. In this period there were 8,883 office appointments, during which 2,708 local anesthetics were administered for purposes of cavity preparation upon vital teeth. The age range of patients anesthetized was 5½ to 85 years; there were 840 male patients (31 per cent) and 1,868 female patients (69 per cent).

OBSERVATIONS

One hundred forty (5.1 per cent) of the 2,708 consecutive anesthetics were found to be incomplete or of abnormally short duration. Thirty-two (1.2 per cent) of the 2,708 gave minor toxic reactions. Incomplete or abnormal anesthetics occurred most frequently with mandibular injections, while the toxic manifestations appeared twice as frequently, percentage-wise, among the subperiosteal injections as in either of the other types (Table I).

It is increasingly evident that the incomplete anesthetics are most prone to occur in the mandibular injections. The toxic manifestations occurred in the preliminary study, as well as in this, in greater percentage among the subperiosteal injections (Table II).

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*NPC, procaine-hydrochloride 2 per cent, pontocaine 0.15 per cent, Cobefrin 1:10,000, in Carpules.

TABLE I

ANESTHETIC TYPE	PER CENT INCOMPLETE	PER CENT TOXIC
Mandibular	9.8	0.8
Tuberosity	1.7	0.7
Subperiosteal	1.5	1.8

TABLE II

ANESTHETICS BY TYPE

TYPE	ANESTHETICS		INCOMPLETE		TOXIC REACTIONS	
Mandibular	1,168	43%	115	82%	10	31.0%
Tuberosity	575	21%	10	7%	4	12.5%
Subperiosteal	965	36%	15	11%	18	56.5%
Total	2,708	100%	140	100%	32	100%

There was no significant difference observable according to sex. The percentages of incomplete anesthetics by sex are fairly close to the percentage of total anesthetics administered by sex, and those of toxic manifestations are identical (Table III).

TABLE III

ANESTHETICS ACCORDING TO SEX

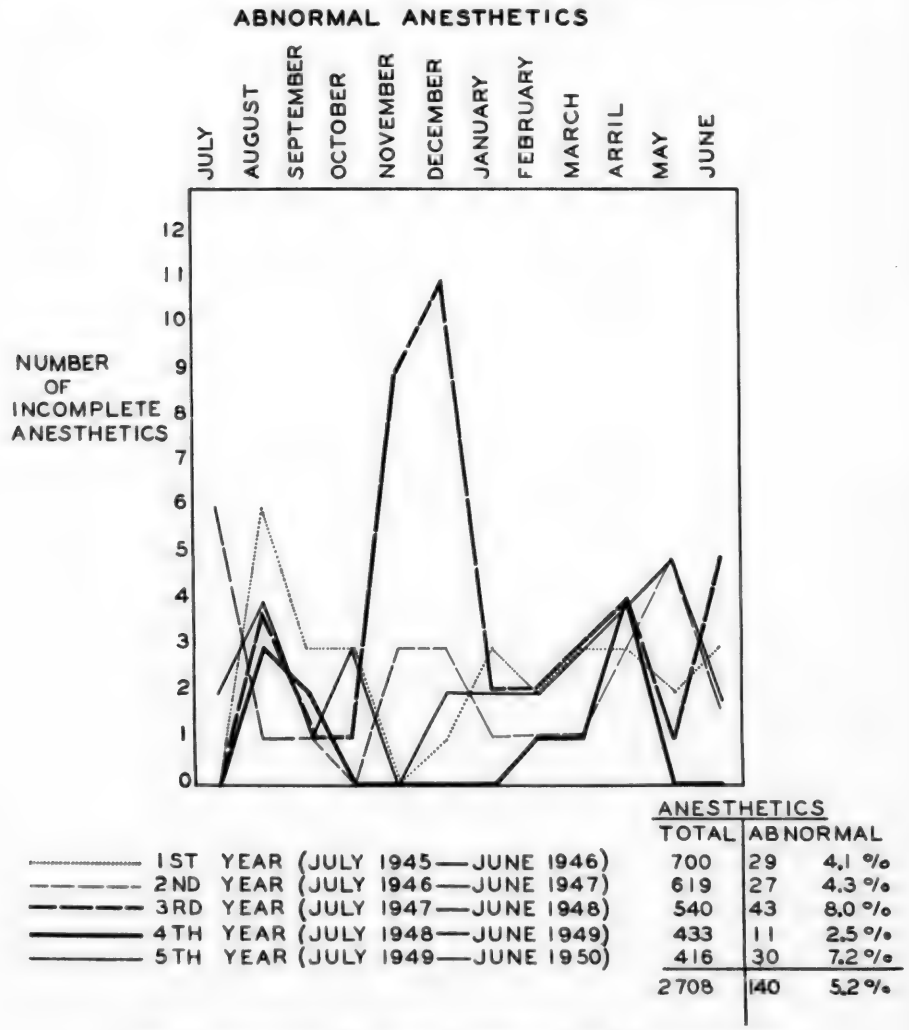
SEX	ANESTHETICS		INCOMPLETE ANESTHESIA		TOXIC REACTIONS	
Males	840	31%	35	25%	11	31%
Females	1,868	69%	105	75%	21	69%
Total	2,708	100%	140	100%	32	100%

All weather data have been included upon the monthly graphs for purposes of study, that is, wind direction, maximum temperature, minimum temperature, barometric pressure, relative humidity at 4:30 A.M. and at noon, and precipitation.

Data in the previous report[1] give some support for the belief that there might be a seasonal relationship in abnormal and in toxic reactions. However, the 5-year data indicate no correlation by season for either the abnormal anesthetics (Fig. 1) or the toxic reactions (Fig. 2). The table relating to prevailing winds showed no correlation in the previous study, and is omitted from this study.

Fig. 3 is a summary: It represents the totaled degrees of plus or minus departures of the daily mean temperatures from the daily normal temperatures for 13 days before the day of the anesthetic recorded, and for 7 days after, in order to achieve a broad base. Throughout this investigation it has become increasingly apparent that the change in temperature, not the degree of temperature, has been the predominantly important factor in bringing about abnormal and toxic manifestations. The abnormal or incomplete anesthetics appear in connection with temperature peaks and are more noticeable when preceded by a series of peaks. This is rather clearly demonstrated in

Fig. 3. The lower temperatures between peaks are referred to as polar infalls, and it has been believed for some time that the toxic manifestations occurred in association with these sharp polar infalls. Fig. 3 appears to afford more clear evidence at this time that the toxic manifestations are thus associated than was apparent at the end of the 2-year period.

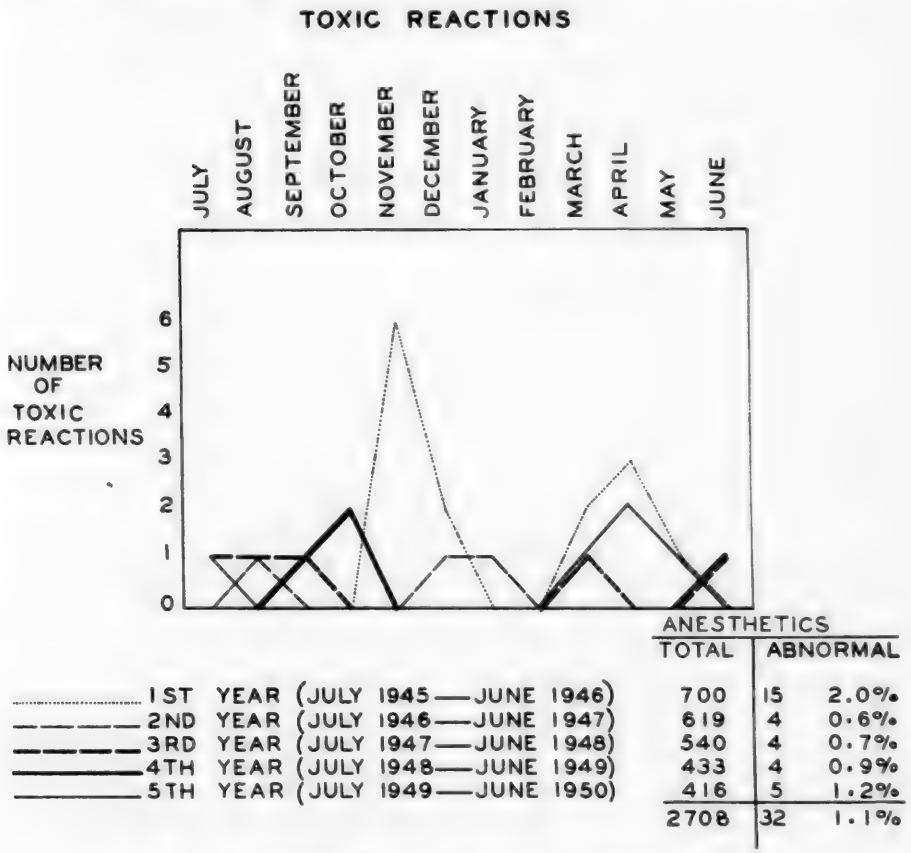


ANESTHETICS		
TOTAL	ABNORMAL	
700	29	4.1 %
619	27	4.3 %
540	43	8.0 %
433	11	2.5 %
416	30	7.2 %
2708	140	5.2 %

Fig. 1.—Comparison of abnormal anesthetics for 5 12-month periods.

The averaged temperature-range variations and averaged humidity variations which were included in the preliminary study have been omitted here because they were found always to be in harmony with the temperature variations as expressed in Fig. 3.

The barometric pressure variations previously were found to be so slight as to present a graph rapidly approaching a straight line as they were averaged for larger and larger numbers of cases.*



	ANESTHETICS		
	TOTAL	ABNORMAL	
1ST YEAR (JULY 1945—JUNE 1946)	700	15	2.0%
2ND YEAR (JULY 1946—JUNE 1947)	619	4	0.6%
3RD YEAR (JULY 1947—JUNE 1948)	540	4	0.7%
4TH YEAR (JULY 1948—JUNE 1949)	433	4	0.9%
5TH YEAR (JULY 1949—JUNE 1950)	416	5	1.2%
	2708	32	1.1%

Fig. 2.—Comparison of toxic reactions for 5 12-month periods.

DISCUSSION

In my opinion, the previously stated belief that unusual experiences with local anesthetics are directly related to environmental temperature change has become increasingly sound. It is highly desirable that investigators in other parts of the country undertake similar investigations, since it is entirely possible that weather changes may affect anesthetic reactions differently in other sections. Here in Southern California the temperature changes appear to be the deciding factors; in geographic areas of extreme barometric changes these latter might well show a more demonstrable correlation.

*In one instance in another study which covered a 12-year period and included a similar number of abnormalities, the barometric pressure graph, which was in that case used on a 30-day base, was an almost straight horizontal line.[14]

It has been shown that under environmental temperature changes the distribution of the blood deviates from the normal, becoming predominantly peripheral in distribution with a sudden rise in temperature, or predominantly in the splanchnic beds with a sudden drop in temperature, and that there is a relative anoxia in the tissues affected by reduced blood supply.[3]

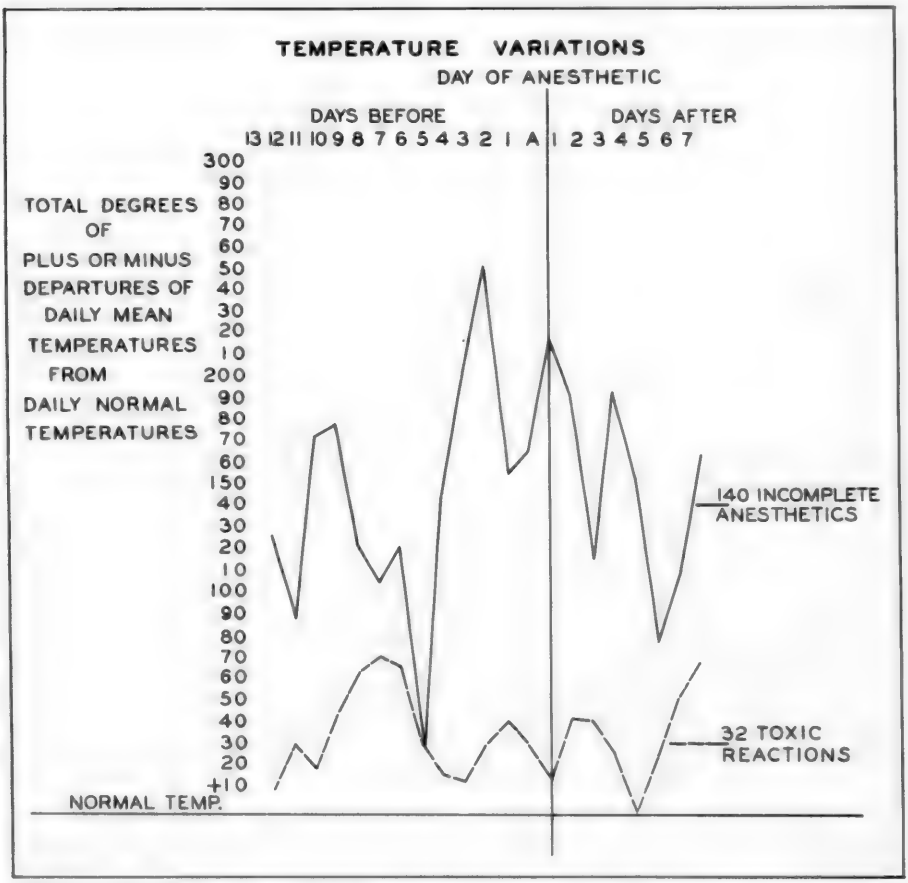


Fig. 3.—Summaries of average conditions preceding incomplete anesthetics and toxic manifestations for a period of 13 days before the date of occurrence and for the succeeding 7 days (2,708 consecutive anesthetics; 140 incomplete anesthetics, 5.1 per cent; 32 toxic reactions, 1.2 per cent).

Theories of the action of local anesthetics upon the nerve tissue, whether the lipoid theory[15, 16] or the enzymatic theory of Rapp,[17] are predicated upon the neutralization of the acid linkage of the procaine molecule by the buffers of the tissue.

From the evidence presented, it seems apparent that the anesthetic reactions noted are due to changes in the patient's organic state, caused by temperature change in the weather. The preponderance of incomplete anesthetics

among the mandibular injections may be explained by the fact that the injection is made into a relatively less vascular area. With the increased distribution of the blood supply to the peripheral tissues, there would be a lessened buffering action available in the nerve tissue itself. The tuberosity and the subperiosteal injections are made in more vascular areas, and there would be a greater possible modification of the solutions by the blood supply in the area.[1]

SUMMARY

1. Of 2,708 consecutive local anesthetics administered for cavity preparation upon vital teeth, 140 (5.1 per cent) gave incomplete pulp anesthesia or anesthesia of very short duration, and occurred in association with temperature peaks.

2. In this series of anesthetics, 32 (1.2 per cent) gave minor toxic manifestations and occurred in association with temperature dips.

3. The change in environmental temperature appears to be the causative factor for these abnormal anesthetic reactions.

4. Toxic manifestations did not occur in cases of incomplete anesthesia, and vice versa. This may be explained by the fact that it is a temperature drop producing the toxic reactions and a temperature rise producing the incomplete anesthetic.

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WEATHER CONDITIONS AFFECTING PATIENTS' REACTIONS TO LOCAL ANESTHETICS

III. A Report of 1,103 Consecutive Cases, Using Lidocaine

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PREVIOUS papers have reported 5 years' experience with the effect of weather conditions on patients' reactions to one local anesthetic solution used in operations upon vital teeth.[1, 2] Reports by Lloyd and Blythe[3] and by Gruber[4] on a new local anesthetic agent differing materially from procaine and its allied group suggested repeating these original studies using the new anesthetic solution* under identically the same conditions as previously reported. These data comprise the present preliminary report.

MATERIALS AND METHODS

During the 3-year period of this study (Sept. 5, 1950, to Sept. 4, 1953) there were 1,103 local anesthetics administered for purposes of cavity preparation upon vital teeth. The age range of the patients was from 9 years to 75 years; there were 294 (27 per cent) male patients, and 809 (73 per cent) female patients.

The same formula of solution was used in every case. As in previous reports,[1, 2] an anesthetic has been designated abnormal when there was incomplete anesthesia of the dental pulp or when the duration of anesthesia was unusually short. In many cases a second injection has been satisfactory in producing the desired results. In a few cases it has been impossible to obtain satisfactory results at all during that appointment. Toxic manifestations have been noted whenever tachycardia, shortness of breath or pallor, or sensation of weakness in the knees was reported. Data were plotted on the monthly graphs exactly as described in previous reports,[1, 2] but for this report nonpertinent data are omitted. The only difference therefore in this and the previous studies is the different anesthetic solution used.

OBSERVATIONS

Forty-one (3.7 per cent) of the 1,103 consecutive anesthetics were found to be incomplete or of very short duration (7.4 per cent of the mandibulars, 1.4 per cent of the tuberosities, and 0.5 per cent of the subperiosteals). Only 2 (0.18 per cent) of the 1,103 anesthetics gave minor toxic reactions. The incomplete anesthetics occurred most frequently with the mandibular injections. The toxic manifestations bore no significant relationship to the type of injection, since there were only 2 in the total series of anesthetics (1 mandibular and 1 subperiosteal).

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*Lidocaine HCl (Xylocaine HCl) 2 per cent, epinephrine 1:100,000, supplied in Carpules of 1.8 c.c.

In Table I the relations of the various types of anesthetics to the whole number are shown, and the percentages of incomplete distributed according to types of injections. As in the previous studies,[1, 2] the greatest number of incomplete anesthetics occurred in mandibular injections. The small number of toxic manifestations has no significance in relation to type of anesthetic, as stated previously.

TABLE I

ANESTHETICS BY TYPE

TYPE	ANESTHETICS		INCOMPLETE		TOXIC REACTIONS	
Mandibular	471	43%	35	85%	1	50%
Tuberosity	275	25%	4	10%		
Subperiosteal	357	32%	2	5%	1	50%
Total	1,103	100%	41	100%	2	100%

Table II shows that there is little difference in reaction according to sex. The incomplete anesthetics were in approximately the same relationship according to sex as the total number of anesthetics administered.

TABLE II

ANESTHETICS ACCORDING TO SEX

SEX	ANESTHETICS		INCOMPLETE ANESTHESIA		TOXIC REACTIONS	
Males	294	27%	8	20%	1	50%
Females	809	73%	33	80%	1	50%
Total	1,103	100%	41	100%	2	100%

Fig. 1 is a summary. It presents the averaged conditions of temperature change preceding the incomplete anesthetics and the toxic manifestations for a period of 13 days before the date of occurrence and for the succeeding 7 days. In this way a broad base is achieved. In this figure are given the totaled degrees of plus or minus departures of the daily mean temperatures from the daily normal temperatures. It has previously been noted that the incomplete anesthetics appeared to occur in connection with temperature peaks, and were most prone to occur when preceded by a series of peaks with the intervening polar infalls. As increased numbers of cases are graphed, this becomes increasingly clear.

The composite curve for the two toxic manifestations would have no significance were it to be considered alone. In the light of previously reported experiences,[1, 2] however, it is significant to note that the days of occurrence of the toxic manifestations fall on the lowest point of the very moderate curve.

This temperature change has been found to be the pertinent demonstrable factor in this area of Southern California. While the originally considered weather factors of wind direction, maximum temperature and minimum temperature spread, barometric pressure, relative humidity, and precipitation have been continued on the monthly graphs, they have been found to be in-

significant in that either no relationship can be found, or they are simply in complete harmony with the temperature variations expressed in Fig. 1. The seasonal concept of abnormal reactions was found to be untenable in the preceding 5-year study.[2]

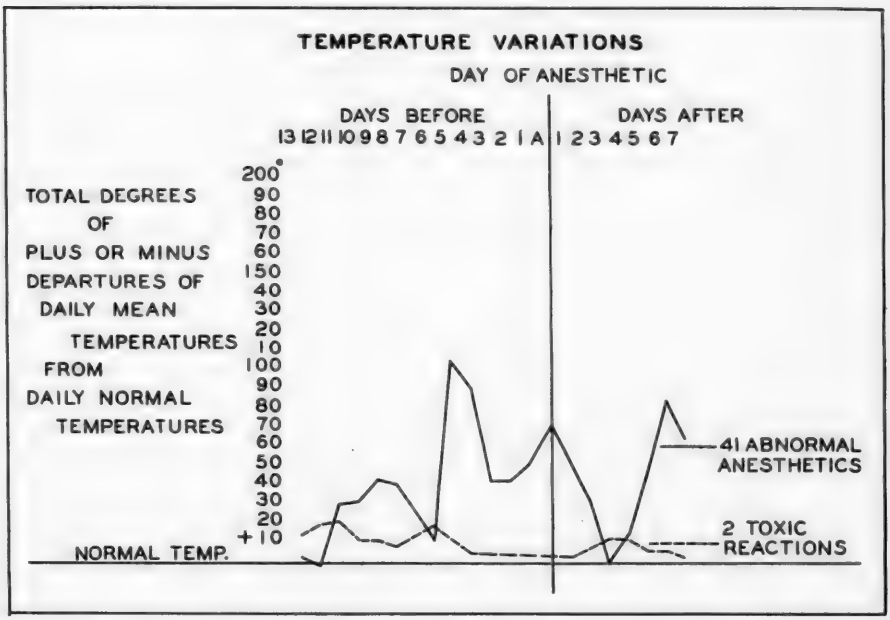
TEMPERATURE VARIATIONS
DAY OF ANESTHETIC
DAYS BEFORE
DAYS AFTER
13 12 11 10 9 8 7 6 5 4 3 2 1 A 1 2 3 4 5 6 7
TOTAL DEGREES OF PLUS OR MINUS DEPARTURES OF DAILY MEAN TEMPERATURES FROM DAILY NORMAL TEMPERATURES
200 90 80 70 60 150 40 30 20 10 100 90 80 70 60 50 40 30 20 +10
NORMAL TEMP.
41 ABNORMAL ANESTHETICS
2 TOXIC REACTIONS

Fig. 1.—Summaries of average conditions preceding incomplete anesthetics and toxic manifestations for a period of 13 days before the date of occurrence and for the succeeding 7 days (1,103 consecutive anesthetics; 41 incomplete anesthetics, 3.7 per cent; 2 toxic reactions, 0.18 per cent).

DISCUSSION

In reviewing this 3-year survey with this particular anesthetic solution, it is evident that there may be some slight advantage in one anesthetic solution over another in so far as efficacy of producing anesthesia occurs. It is of relatively small significance, however, when compared with the previously reported 5-year survey with another solution.[2] This preliminary report would indicate a marked reduction of toxic manifestations displayed by this substitute anesthetic solution. The important factor, it appears, is that the environmental weather temperature change in Southern California may produce deviations from normally anticipated anesthetic reactions.

Dental and medical colleagues have indicated to me that they have experienced similar difficulties in inducing complete local anesthesia under certain conditions. Cartwright and Rogers[5] reported unsatisfactory or incomplete anesthesia in 53 of 1,021 obstetrical patients given spinal anesthesia routinely for vaginal deliveries.

Individuals have different capacities for accommodation to environmental change.[7] It would appear from these studies that the repeated impacts of environmental temperature change have exceeded the capacity of the individual to accommodate when an unusual anesthetic reaction is experienced.

It has been noted that certain individuals encountered in this practice of general dentistry have relatively low capacities to meet these impacts of temperature change and present difficulties to anesthesia except under what subsequently* appears to have been very favorable conditions. Other individuals are able to accommodate successfully in most instances but under conditions of fatigue or other organic depressants are unable to meet the demands of marked environmental temperature change.

It has appeared that the probable incomplete anesthetic patients lie among those giving some evidence of inadequate circulatory mechanisms with a tendency toward water retention in the tissues. This thought is speculative at this time, but points up the necessity for more complete investigation of patients' physical states. With the recognition that environmental temperature changes do have an influence upon patients,[6-20] the practical question is, what can be done? Unfortunately, it seems such investigation exceeds the possibilities of investigation in private dental practice.

SUMMARY

1. Among 1,103 consecutive local anesthetics administered for cavity preparations upon vital teeth, 41 (3.7 per cent) gave incomplete pulp anesthesia or anesthesia of very short duration, and occurred in association with temperature peaks.

2. In this series of anesthetics only 2 (0.18 per cent) gave minor toxic manifestations. These occurred in assoociation with temperature dips.

3. The temperature change, not degree either of heat or of cold, appears to be the causative factor for these abnormal anesthetic reactions.

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*At the end of each month a weather summary, supplied by the Weather Bureau of the United States Department of Commerce at Los Angeles, has been used to mark on the graph the weather changes shown. This is received approximately the fifth day of the succeeding month.

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SOME FACTORS AFFECTING THE DIMENSIONAL STABILITY OF THE AG-SN-(CU-ZN) AMALGAMS*

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INTRODUCTION

ALTHOUGH an enormous amount of experimental work has been done on the Ag-Sn-(Cu-Zn)† amalgams,[1-6] no mechanism has been proposed which will satisfactorily explain the initial contraction, subsequent expansion, and final contraction which occur during the first twenty-four hours after amalgamation. Various investigators have attempted to explain these changes in terms of mercury content of the amalgam system. Their difficulty may have resulted from the failure to differentiate between total mercury and combined mercury in this system. It is probable that in the hardening of the heterogeneous mass, not all the mercury is in the combined form. Chemical analysis of the amalgam would not distinguish between combined and uncombined mercury. Furthermore, photomicrographic evidence[7] indicates that the rate of attack on the alloy particles by mercury may vary widely depending upon the conditions under which the specimen was prepared. The possible diffusion of uncombined mercury and its subsequent reactions with the alloy may account for the dimensional changes observed in the hardened amalgam.

This investigation was undertaken to determine (1) whether uncombined mercury was present after the amalgam had hardened; (2) whether the uncombined mercury, if present, continued to take part in any reaction; and (3) whether the dimensional changes which occur in hardened amalgam could be correlated with this reaction.

EXPERIMENTAL APPROACH AND PROCEDURES

X-ray diffraction methods cannot be used to detect the presence of free or uncombined mercury in an amalgam at room temperature since the mercury, being a liquid at this temperature, does not have a crystalline structure. If the mercury is chilled below its solidification point, however, typical lines representing mercury are detected by diffraction methods. Therefore, if uncombined mercury is present in amalgam immediately after hardening, it should be possible to determine its presence by x-ray investigation of amalgam specimens held below the freezing temperature of mercury. Furthermore, it should be possible

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**Lieutenant (DC) USN, stationed at the National Bureau of Standards.

†Cu-Zn is placed in parentheses because these elements are not always present in the previously mentioned amalgams.

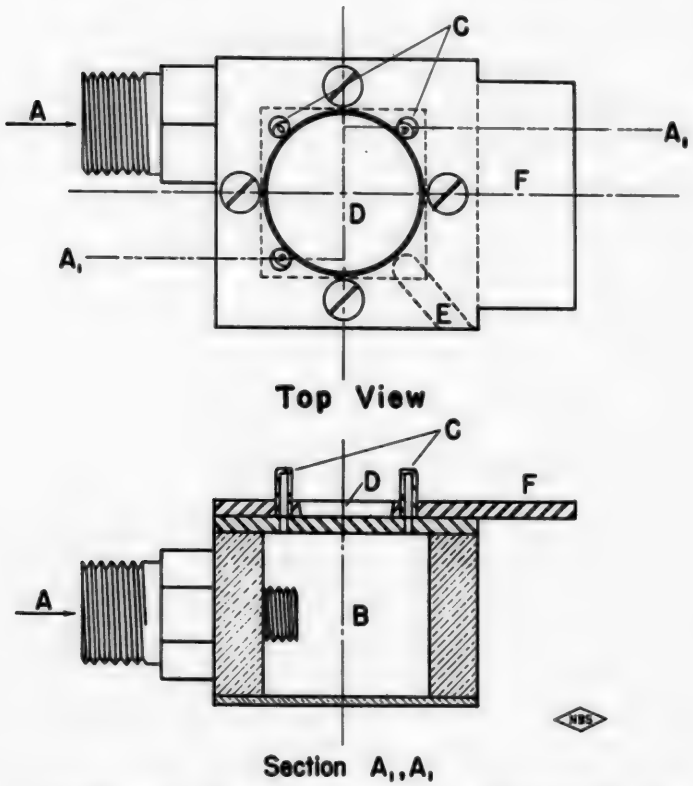

Fig. 1.—Amalgam mold and specimen holder for low-temperature x-ray diffraction studies. Holder enclosed in a cylindrical box. Specimen retained in holder and alternately cooled to −125° C. by liquid nitrogen and warmed to 37° C. by a wire-wound resistor regulated with a variable autotransformer. *A*, Liquid nitrogen intake; *B*, liquid nitrogen chamber; *C*, jets; *D*, specimen; *E*, thermocouple rest; *F*, arm fastened to goniometer shaft.

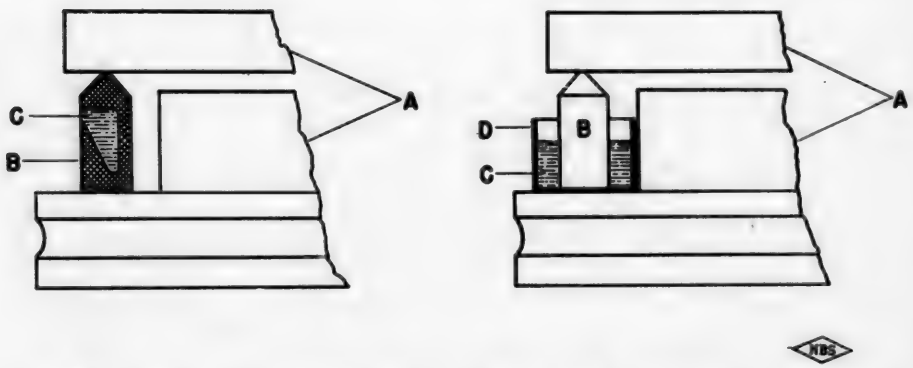

Fig. 2.—Measurement of dimensional change of fully hardened amalgam specimens after addition of mercury. Mercury added internally and externally. *A*, Dental interferometer; *B*, specimen; *C*, mercury; *D*, iron cup.

to study the rate of disappearance of the uncombined mercury with time by periodic examination of the specimen at temperatures below the freezing point of mercury.

X-ray diffraction patterns of the amalgams at low temperatures were obtained from specimens which were manually condensed, using standard dental procedures, in a stainless steel mold and specimen-holder combination (Fig. 1). Immediately after condensation, the amalgam specimens were frozen by introducing liquid nitrogen into the chamber beneath the specimen. An iron-constantan thermocouple was inserted in the specimen holder near the specimen for temperature determination.

Three commercial amalgam alloys were used in the investigation. Amalgams were produced by triturating the alloys with mercury in a mechanical amalgamator using the mercury-alloy ratio of 2 to 3 for alloys 1 and 2, and a ratio of 2 to 3.6 for alloy 3.

A Philips high-angle spectrometer goniometer[8] was used to obtain chart records of the copper K alpha diffraction. The diffraction unit was operated at 35 KVP and a tube current of 2θ milliamperes. The angular speed of the scanning arm was one degree per minute and the chart speed was 0.5 inch per minute.

X-ray diffraction characteristics were obtained with the specimen and its holder maintained at the constant reduced temperature (–125° C.) during the making of chart records in a 20 range of 80° to 28°. The specimen was then warmed to mouth temperature (37° C.) and aged at that temperature until the next low temperature diffraction chart recording was made.

The x-ray diffraction technics described previously were used also to determine the effect of addition of mercury to fully hardened amalgam specimens. Seven-day-old specimens were immersed in mercury for twelve hours, removed, cooled to –125° C., and examined by x-ray diffraction. The specimens were kept at 37° C. during the next twenty-four hours, except for periodic cooling to –125° C. while diffraction charts were made.

TABLE I

CHEMICAL COMPOSITION OF ALLOYS INVESTIGATED

ELEMENTS	NO. 1 (PER CENT)	NO. 2 (PER CENT)	NO. 3 (PER CENT)
Ag	74.5	70.2	70.1
Sn	25.0	25.6	26.5
Cu	0.0	2.4	3.4
Zn	0.5	1.8	0.0

To examine the effect of uncombined mercury on dimensional changes of hardened amalgam, mercury was added both externally and internally to hardened amalgam specimens and their dimensional changes were measured by means of dental interferometers[9] (Fig. 2). Hardened specimens of amalgam in iron cups were positioned in interferometers. Mercury was added to the cups, and the dimensional changes were observed over a period of fifteen days on two series of specimens held at 21° C. and 37° C., respectively. In other experiments, after the specimens had been positioned in the interferometers

mercury was injected by a hypodermic syringe into a small hole drilled in the specimen. Dimensional changes were then observed over a period of fifteen days. Temperatures were maintained by constant temperature air baths.

In two of the aforementioned experiments the supply of mercury was removed from contact with the specimens after the period of fifteen days and dimensional changes were observed for an additional period of twenty-six days.

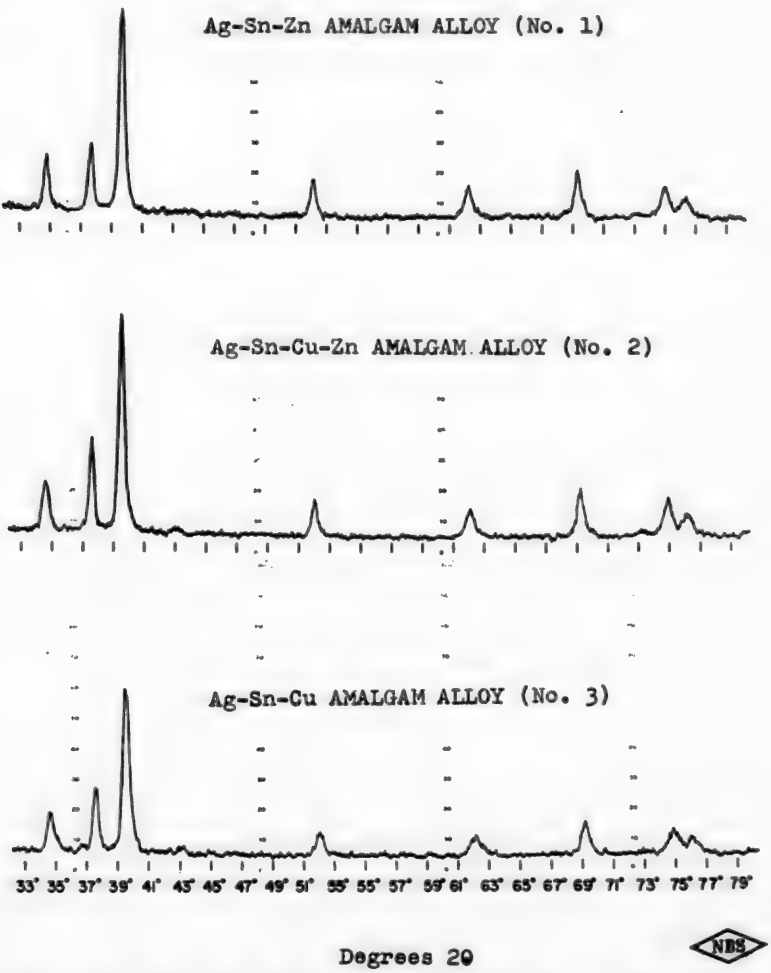
Ag-Sn-Zn AMALGAM ALLOY (No. 1)
Ag-Sn-Cu-Zn AMALGAM ALLOY (No. 2)
Ag-Sn-Cu AMALGAM ALLOY (No. 3)
33° 35° 37° 39° 41° 43° 45° 47° 49° 51° 53° 55° 57° 59° 61° 63° 65° 67° 69° 71° 73° 75° 77° 79°
Degrees 2θ
NBS

Fig. 3.—X-ray diffraction charts (with a scale factor of 4) made at 25° C. The similarity of the charts for the three alloys indicates that none of the lines shown is dependent upon the copper or zinc content of the alloys.

To obtain information on the effect of temperature on the reactions occurring during the hardening of the dental amalgams, dimensional changes of amalgams prepared and observed at various temperatures were also determined interferometrically.

RESULTS

X-Ray Diffraction Examination of the Ag-Sn-(Cu-Zn) Amalgams.—As shown in Table I, one of the three commercial amalgam alloys used in the investigation was a silver-tin-zinc alloy, one a silver-tin-copper-zinc, and the other a silver-tin-copper alloy. Upon examination of their x-ray diffraction patterns

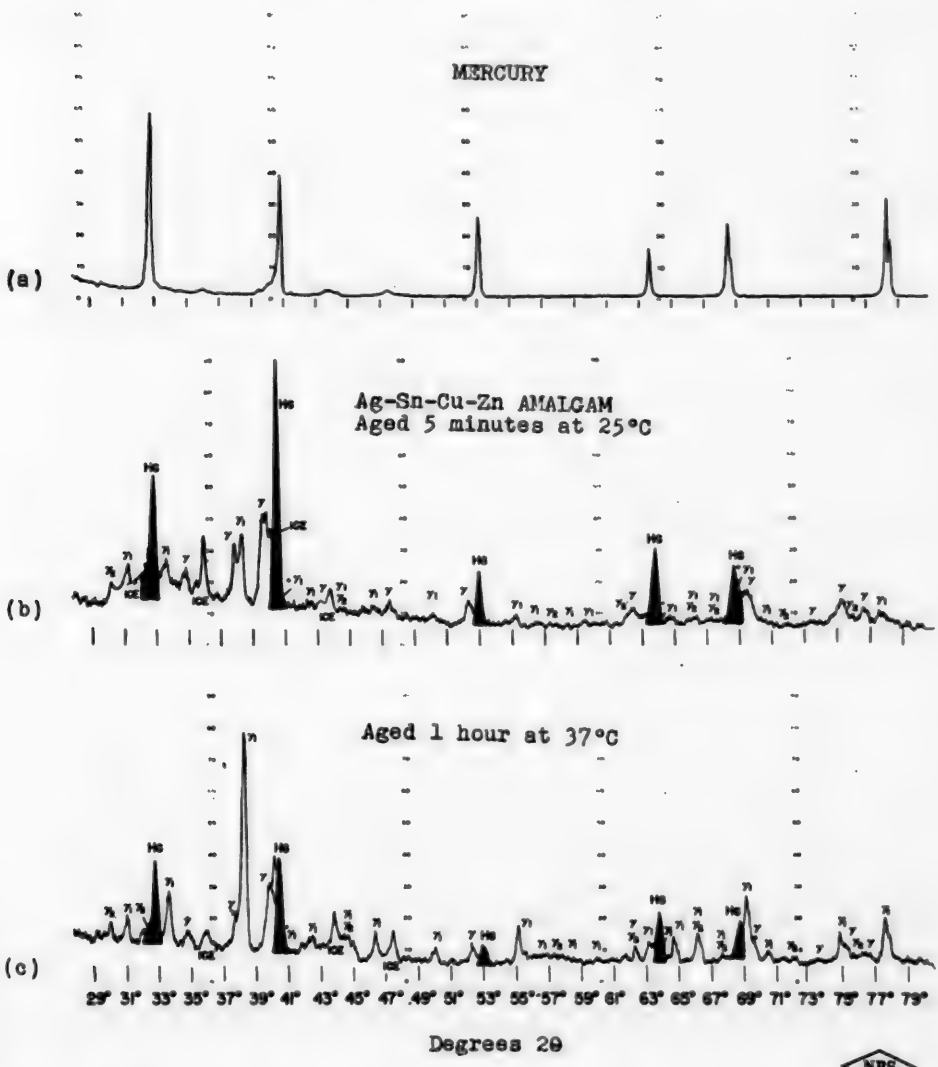
MERCURY
(a)
Ag-Sn-Cu-Zn AMALGAM
Aged 5 minutes at 25°C
(b)
Aged 1 hour at 37°C
(c)
29° 31° 33° 35° 37° 39° 41° 43° 45° 47° 49° 51° 53° 55° 57° 59° 61° 63° 65° 67° 69° 71° 73° 75° 77° 79°
Degrees 2θ
NBS

Fig. 4.—X-ray diffraction charts made at −125° C. Scale factor of 32 used for obtaining the tracings of mercury. Scale factor of 4 used for the remainder of the procedures. Height of the peaks of γ_1 and γ_2 lines hidden by the mercury lines was determined by x-ray diffraction charts made at 37° C. Lines representing ice formation are labeled *"ICE."*

(Fig. 3), it is evident that the variation in copper and zinc content has little effect on the diffraction patterns, since the position of the principal peaks is identical in all three charts.

X-ray diffraction charts of pure mercury at –125° C. show a number of well-defined peaks (Fig. 4, *a*). A specimen of amalgam containing the silver-tin-copper-zinc alloy was made at room temperature (25° C.), placed in position,

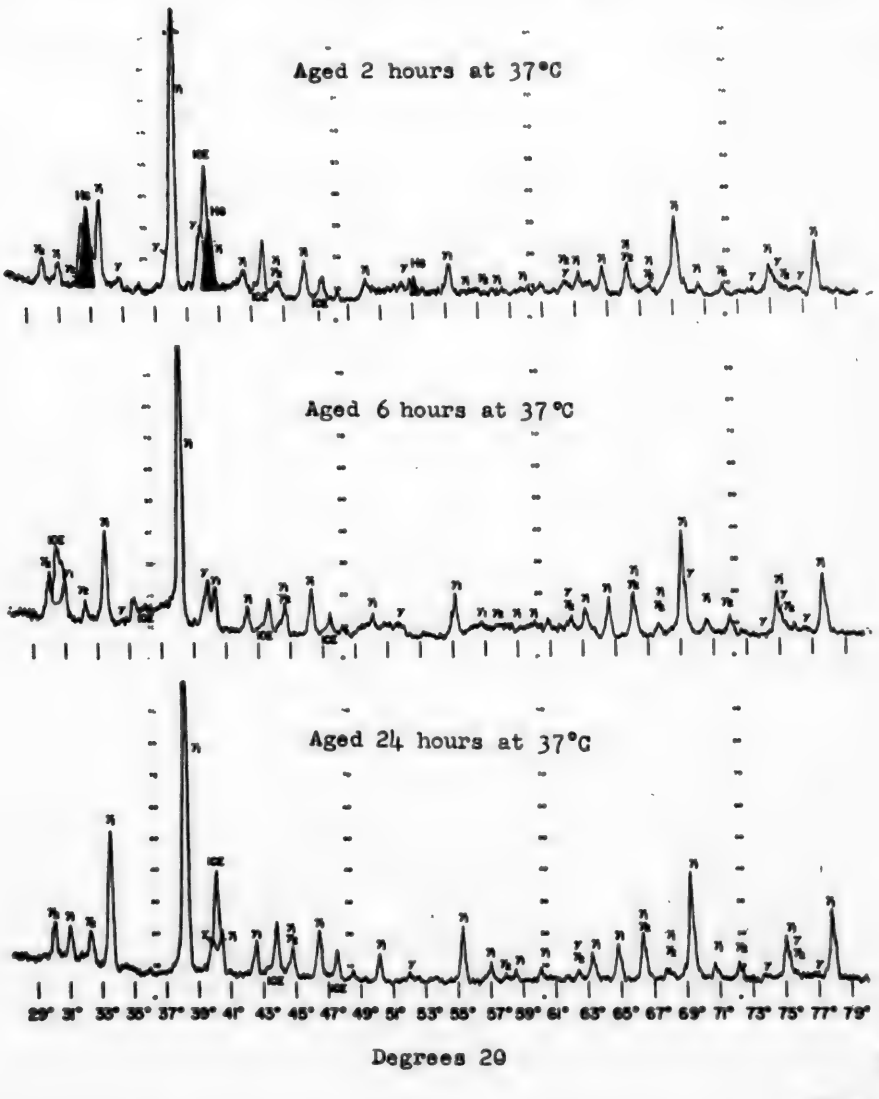

Fig. 5.—X-ray diffraction charts, a continuation of Fig. 4.

and its x-ray pattern determined at –125° C. five minutes after start of mix (Fig. 4, *b*). The characteristic peaks of the uncombined mercury are represented by the solid black areas. As the specimen is aged through longer periods

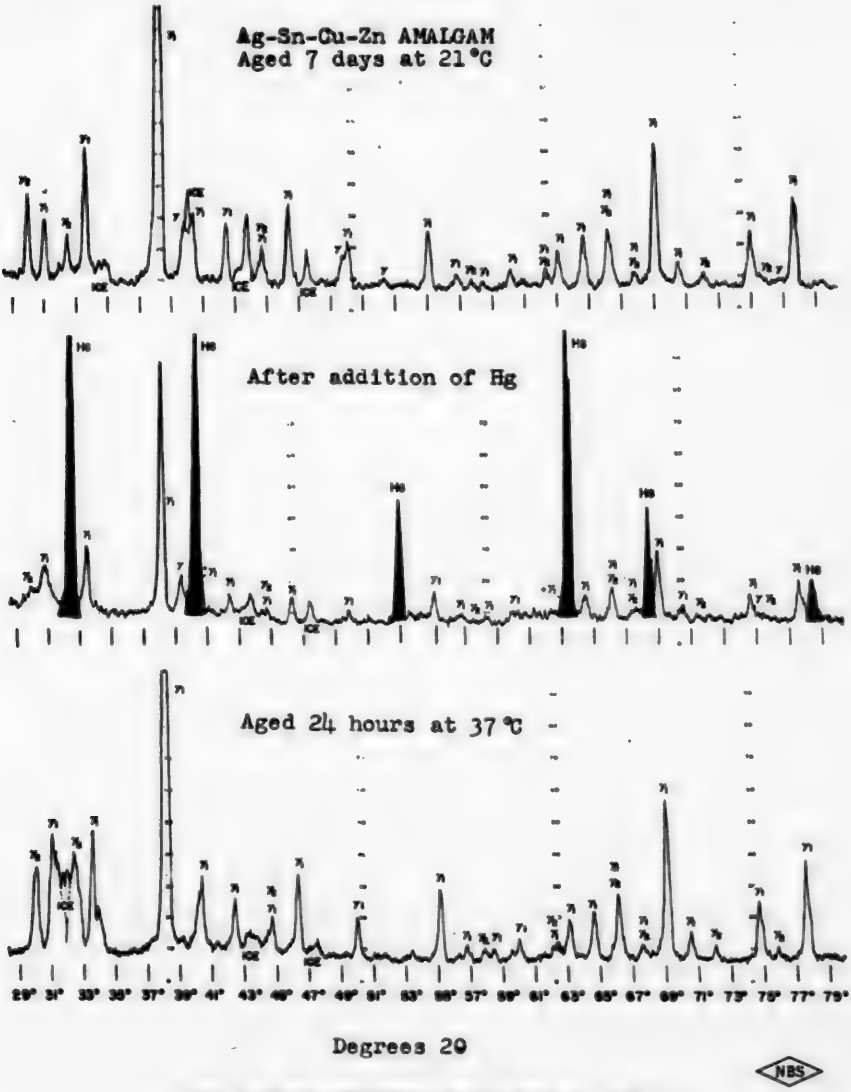

Fig. 6.—X-ray diffraction charts made at –125° C.

of time at 37° C., the characteristic peaks of the uncombined mercury tend to disappear, until after six hours they are scarcely detectable (Fig. 4, *c*, and Fig. 5). This time period corresponds generally to the period during which significant expansion and contraction are commonly observed. Simultaneously with

this reduction in uncombined mercury, there is a disappearance of the lines representing the original alloy (γ)* and an increase in the γ_1 and γ_2 phases (Fig. 4, *b* and *c*, and Fig. 5). This indicates that the disappearance of the free mercury lines was through combination of the mercury with the alloy to form mercury compounds.

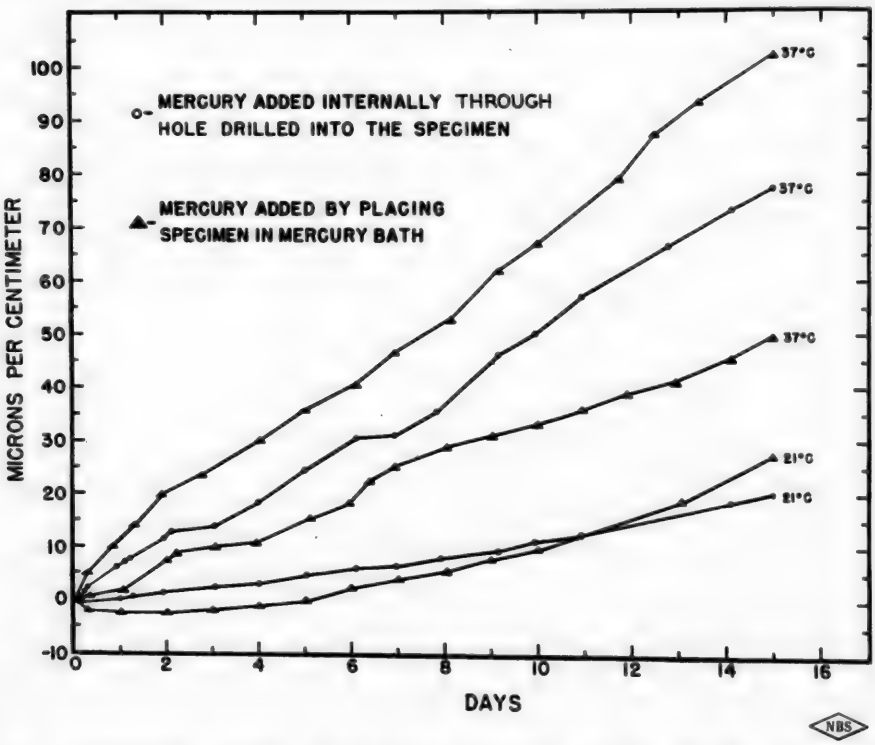

Fig. 7.—Dimensional changes in Ag-Sn-Cu-Zn amalgam (alloy 2) resulting from addition of mercury.

X-ray diffraction charts made periodically at reduced temperatures after the addition of mercury to fully hardened amalgam showed intense mercury lines in the specimens observed immediately after removal from the mercury bath (Fig. 6). These mercury lines were not detected after the specimens had been held at 37° C. for twenty-four hours or at 21° C. for three days. Mercury on the surface of the specimen reduces the intensity of the lines representing the original alloy and γ_1 and γ_2 phases (Fig. 6). After aging for twenty-four hours, the mercury has combined with the original alloy as indicated by the absence of γ and uncombined mercury lines.

*α –original alloy (Ag_3Sn + Cu-Zn)[3, 5, 6]
α_1 –Ag_2Hg_3
α_2 –Sn_7Hg

Consideration was given to the possibility that the intermediate phases in the Ag-Sn-(Cu-Zn) amalgams might have a higher mercury solubility at room temperature than at temperatures below the freezing point of mercury. Under such conditions, crystalline mercury at low temperatures might be formed by rejection from a solid solution which existed at room temperature. However, the disappearance of the lines representing uncombined mercury indicates that such a rejection does not occur to a significant extent, if at all.

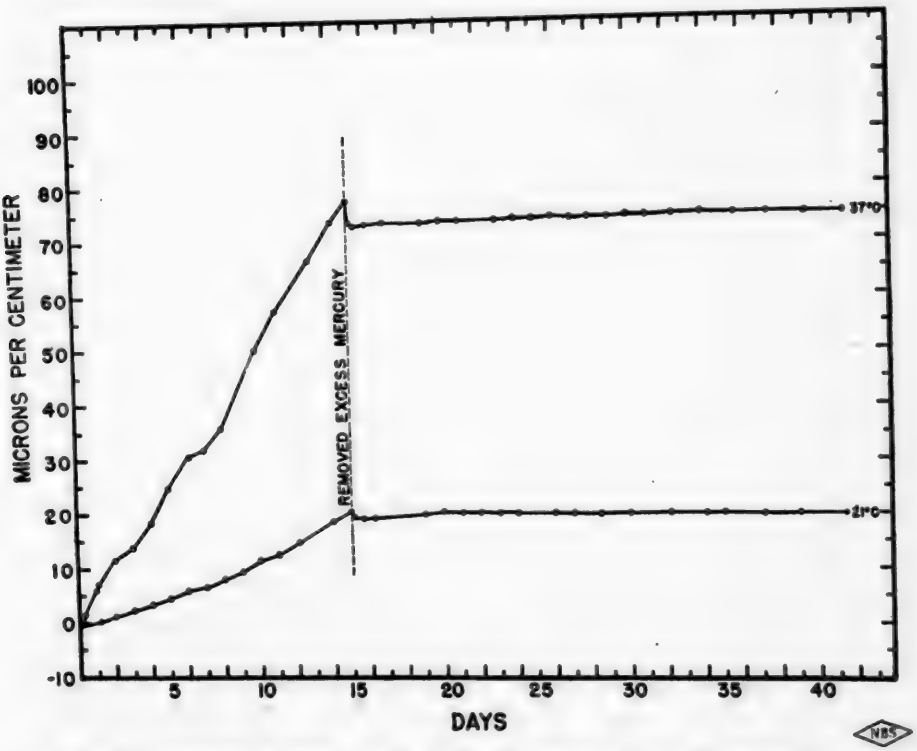

Fig. 8.—Dimensional changes of Ag-Sn-Cu-Zn amalgam (alloy 2) resulting from addition and removal of mercury. 0 Days—Mercury added internally through hole drilled into specimen. 15 Days—Excess mercury removed from hole drilled into specimen.

Dimensional Change Determinations of the Ag-Sn-(Cu-Zn) Amalgams.—The addition of mercury to the hardened specimens produced large expansions whether the mercury was added internally or externally (Fig. 7). Specimens maintained at 37° C. expanded much more than those maintained at 21° C. The great difference in expansion between the two specimens placed in mercury baths at 37° C. may result from imperfections or other variations in the individual specimens which affect the rate of diffusion of mercury into the amalgam. Only very slight dimensional changes occurred after removal of the supply of excess mercury from hardened specimens (Fig. 8).

Amalgam specimens prepared and maintained at either 21° C. or 37° C. showed a rapid initial contraction followed by some expansion during the first

twenty-four-hour period. Average values for dimensional changes are plotted against time in Figs. 9, 10, and 11. Ag-Sn-Zn amalgams prepared at 21° C. and maintained at 37° C. gave an over-all shrinkage, whereas those prepared and maintained at 21° C. gave an over-all expansion (Fig. 9 and Table II). Ag-Sn-Cu-Zn amalgams prepared and maintained at 37° C. also had greater initial

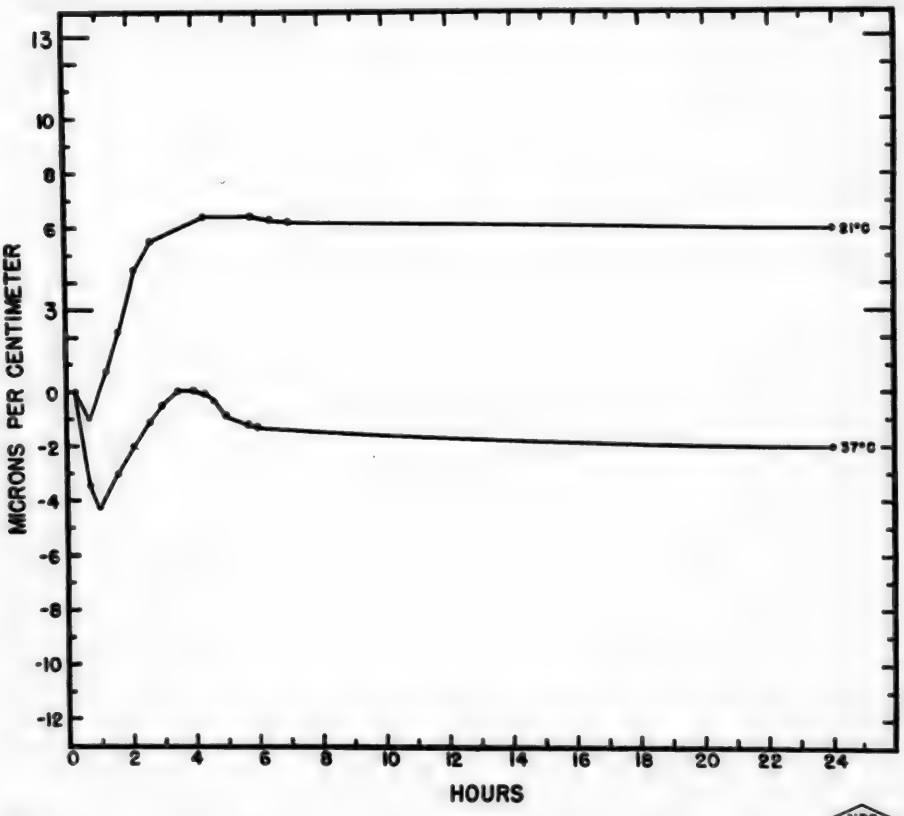

Fig. 9.—Comparison of dimensional changes of Ag-Sn-Zn amalgam (alloy 1) at 21° C. and at 37° C. Specimens prepared at 21° C.

contraction, lower maximum expansion, and greater over-all contraction than those prepared and maintained at 21° C. (Fig. 10 and Table II). Ag-Sn-Cu-Zn amalgams initially observed at 21° C. and then subjected to a temperature increase at 37° C. shrank more than those maintained at 21° C. during the observation period (Fig. 11 and Table II). Specimens held at 37° C. and then subjected to a temperature decrease to 21° C. shrank less than those maintained at 37° C. (Fig. 11 and Table II). Ag-Sn-Cu amalgams observed at 37° C. also shrank more than those observed at 21° C. (Table II).

Averages of the differences in dimensional changes of the Ag-Sn-(Cu-Zn) amalgams at 21° C. and 37° C. are given in Table II, together with their standard errors.

The standard error, in each case, depends on the number of specimens and on the precision of a single measurement. The latter was evaluated, separately for each period of hardening, by pooling the variances, obtained for all amalgams, of the measurements made at that setting time.

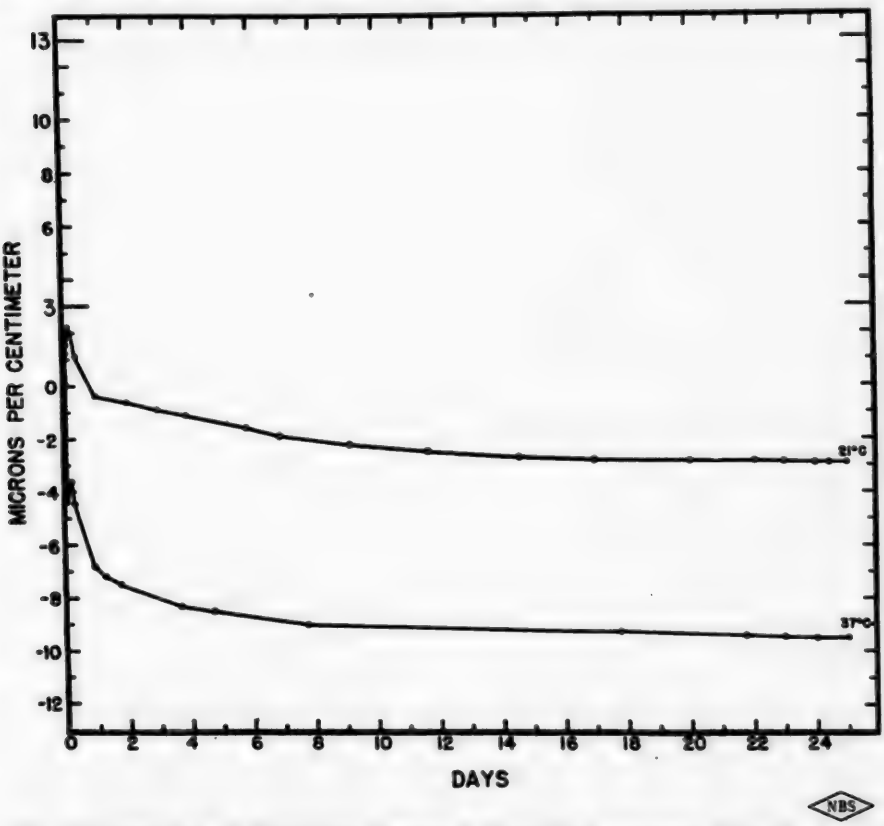

Fig. 10.—Comparison of dimensional changes of Ag-Sn-Cu-Zn amalgam (alloy 2) at 21° C. and at 37° C. Specimens prepared at 21° C. and 37° C., respectively.

The fiducial observation (zero reading) taken ten minutes after start of mix yielded a significantly lower expansion or greater contraction value over a twenty-four-hour period than the fifteen-minute fiducial observation (Table II). This variation results from the fact that a larger portion of the initial shrinkage is observed when the ten minutes fiducial reading is used.

DISCUSSION

The observations of dimensional changes, along with the x-ray diffraction evidence that uncombined mercury is present in the amalgam, tend to substantiate the theory that the mechanism of dimensional change is associated with the presence of the uncombined mercury.

The following mechanism is postulated to explain the physical phenomena observed: The initial shrinkage always observed results from the formation of

compounds of mercury and alloy having a smaller volume than the sum of the original volumes of the alloy and mercury. The subsequent expansion which is observed results from the diffusion into new areas of uncombined mercury entrapped in spaces throughout the amalgam. The reaction of this uncombined mercury with existing phases or with residual alloy to form compounds of lesser volume would then account for the contraction which follows the expansion of the amalgam.

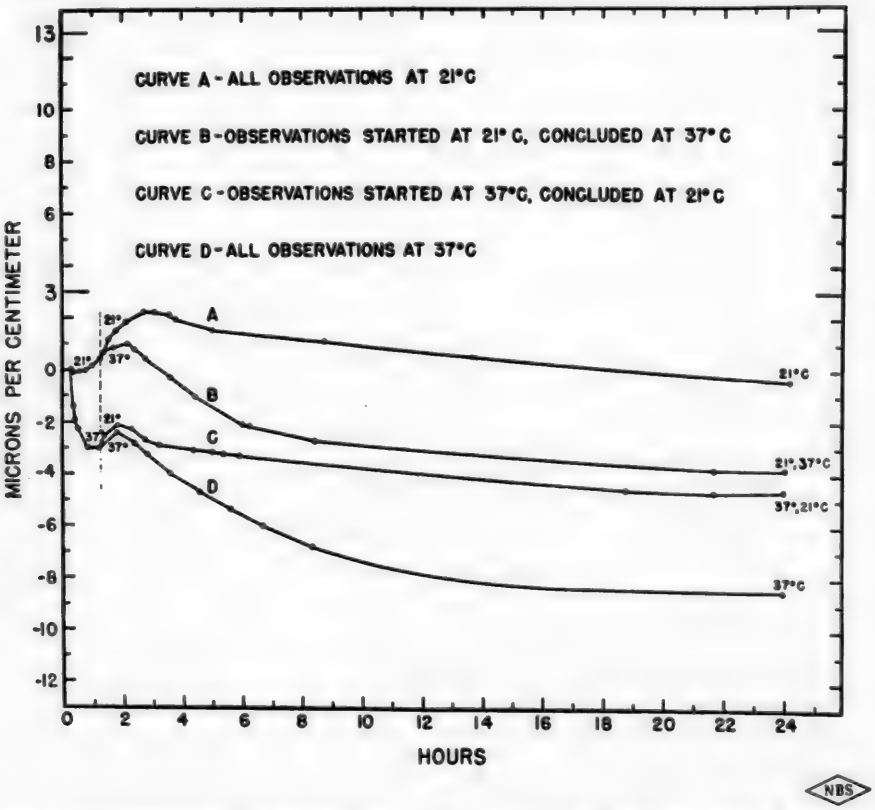

Fig. 11.—Comparison of dimensional changes of Ag-Sn-Cu-Zn amalgam (alloy 2) at 21° C. and at 37° C. Specimens prepared at 21° C.

This mechanism would account for the dimensional changes which occurred on the addition of mercury to hardened amalgam. When mercury is added to hardened amalgam the diffusion of this mercury throughout the specimen would produce the large expansion observed. The slight shrinkage observed immediately after the removal of the supply of mercury could result from the combination of free mercury in the specimen with existing phases or with residual alloy.

The greater shrinkage of amalgam mixed and maintained at 37° C. rather than at 21° C. may result from a more rapid chemical combination and disappearance of the uncombined mercury at elevated temperatures.

The proposed mechanism would also account for the shrinkage of amalgam which occurs from overtrituration or excessive working during condensation in that either of these treatments would tend to reduce the amount of uncombined mercury by either furnishing new surfaces for reaction with the mercury or by actual mechanical removal of the mercury. These treatments would also tend to distribute the uncombined mercury present more uniformly throughout

TABLE II

DIMENSIONAL CHANGES AND THEIR STANDARD ERRORS FOR AG-SN-(CU-ZN) AMALGAMS AT 21° C. AND 37° C.

ALLOY NO.	TEMP. OF OBS.* (° C.)	NO. OF SPECIMENS OBSERVED	FIDUCIAL OBS. 15 MIN. AFTER START OF MIX: INITIAL CONTRACTION (μ/CM.)	MAXIMUM EXPANSION (μ/CM.)	24-HR. CHANGE (μ/CM.)	FIDUCIAL OBS. 10 MIN. AFTER START OF MIX 24-HR. CHANGE μ/CM.	TYPICAL CURVE
1	21	7	−1.3 ± 0.1	+7.0 ± 0.3	+6.2 ± 0.3	+4.3 ± 0.5	Fig. 9
	37	8	−4.1 ± 0.4	+0.0 ± 0.3	−2.1 ± 0.3	−6.2 ± 0.4	
	Difference		2.8 ± 0.5	7.0 ± 0.4	8.3 ± 0.5	10.5 ± 0.6	
2§	21	4	−0.3 ± 0.5	+2.1 ± 0.4	−0.9 ± 0.5		Fig. 10
	37†	4	−4.2 ± 0.5	−3.4 ± 0.4	−7.0 ± 0.5		
	Difference		3.9 ± 0.6	5.5 ± 0.6	6.1 ± 0.6		
2	21	15	−0.3 ± 0.05	+2.0 ± 0.2	−1.0 ± 0.2	−2.6 ± 0.3	Fig. 11
	37	7	−2.6 ± 0.3	−2.0 ± 0.3	−8.6 ± 0.3	−12.6 ± 0.5	Curves
	Difference		2.3 ± 0.3	4.0 ± 0.4	7.6 ± 0.4	10.0 ± 0.6	*A* and *D*
2	21	15	−0.3 ± 0.05	+2.0 ± 0.2	−1.0 ± 0.2		Fig. 11
	21	14	Not observed	+1.3 ± 0.4	−3.4 ± 0.4		Curves
	Changed to 37			0.7 ± 0.5	2.4 ± 0.5		*A* and *B*
	Difference						
2	37	7	−2.6 ± 0.3	−2.0 ± 0.3	−8.6 ± 0.3		Fig. 11
	37	4	Not observed	−1.7 ± 0.4	−4.6 ± 0.4		Curves
	Changed to 21			0.3 ± 0.5	4.0 ± 0.6		*C* and *D*
	Difference						
3‡	21	10	−0.3 ± 0.05	+3.6 ± 0.3	+2.4 ± 0.3	+1.2 ± 0.4	
	37	7	−1.8 ± 0.2	+1.4 ± 0.3	−2.1 ± 0.3	−5.2 ± 0.5	
	Difference		1.5 ± 0.2	2.2 ± 0.4	4.5 ± 0.4	6.4 ± 0.6	

*Specimens prepared at 21° C. unless otherwise noted.

†Specimens prepared at 37° C.

‡Not comparable with alloys Nos. 1 and 2 because of differences in alloy-mercury ratio and mixing time.

§Twenty-four-day period with fiducial observation fifteen minutes after start of mix:
21° C. gave an average of −3.0±0.3 μ/cm.
37° C. gave an average of −9.9±0.3
Difference 6.9±0.4

the amalgam so that there would be fewer mercury-rich areas from which diffusion could take place. On the basis of this explanation, a reduction in size of the alloy particle or a heat treatment which would make the particles more reactive would tend to cause a reduced expansion or a shrinkage of the amalgam.

SUMMARY

Residual or uncombined mercury has been demonstrated by x-ray diffraction studies to be present in amalgams after their initial solidification.

This uncombined mercury disappears during the period when greatest dimensional changes occur in the hardening of the amalgam system.

A possible mechanism for the dimensional changes based on the presence and disappearance of the uncombined mercury is presented.

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A STUDY ON THE PRESSORECEPTIVE SENSIBILITY OF THE TOOTH

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INTRODUCTION

IF THE relatively great density of nerve tissue of the tooth is considered, it is tempting to ask about its functional significance. To attribute to such a rich innervation, exclusively, a pain-receptive function does not seem a satisfactory answer to the question. We think that more appropriately the tooth may be considered as an organ with mixed sensorial functions.

In this paper the tooth is considered as a structure with mixed sensorial functions, its pressoreceptive sensibility being evaluated. It is our aim (a) to locate the receptors of pressure of the tooth, (b) to establish the pressure thresholds of normal and pulpless teeth, and (c) to study the topognosis of dental pressure sensation.

METHOD

Initial experiments done with von Frey's esthesiometers were rejected when a study of the physical properties of these instruments showed considerable variations of the developed force (ab. ± 35%), even if the greatest precautions were taken during the calibration. This is largely due to difficulties in the standardization of the bending moment and to the fact that various physical factors influence the developed force by von Frey's hairs. The use of von Frey's esthesiometers is widely diffused in physiologic studies of pressoreceptors, but our results indicate that von Frey's esthesiometers should not be used in work with highly sensitive pressoreceptors, whenever exactness in threshold determinations is expected.

A spring esthesiometer† was built (Figs. 1*A* and 1*B*): a cone-shaped spiral steel spring *(S)* of 0.30 mm. diameter is subjected to variable tensions. The tension is applied by disk *D* rotating on a common axis *(A)* for spring and disk. Lever *L* transmits the force to the tooth *(T)*. The lever is exactly balanced by the weight *(W)*; thus forces can be applied upward, downward, or horizontally. The apparatus is calibrated in a range of 0 to 6,000 mg. with an accuracy of ± 4 to 5 mg.

For experiments on teeth presenting higher thresholds than 6 Gm. a similar esthesiometer furnished with a spiral spring of 0.45 mm. diameter, calibrated in a range of 0 to 14,000 mg., with a precision of ± 9 to 19 mg. was used. The

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†This instrument was constructed by Mr. Pablo Frigerio, Technical Assistant of the Institute of Physiology.

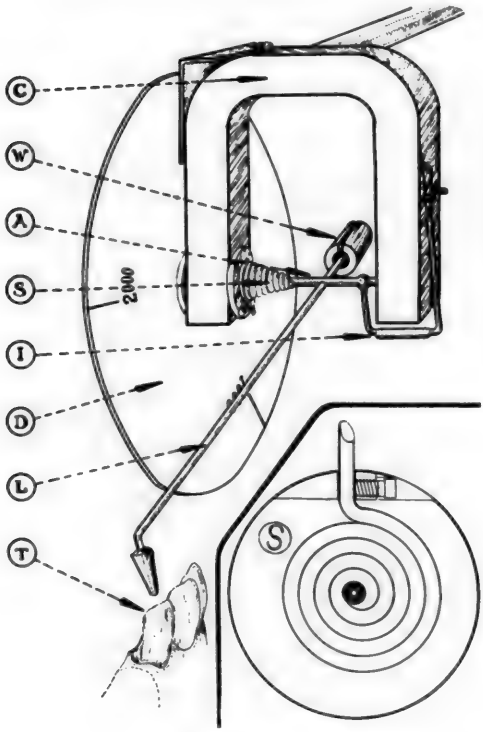

Fig. 1A.

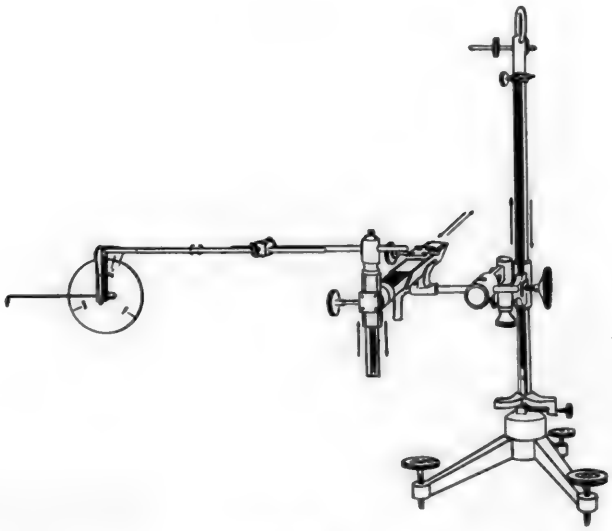

Fig. 1B.

force to be applied to the tooth develops its maximum in less than 2 m. sec., thus providing the operator with an excellent stimulus to prevent sensorial accommodation.

The following procedure was employed: The subject's head is fixed and supported under his chin. Increasing force is applied in direction of the longitudinal axis of the tooth being studied. The subject signals the moment when the sensation of pressure is felt. The minimal force which produces a sensation of pressure is recorded as threshold. At least 3 successive threshold determinations were made: The average was taken as the representative threshold of the particular tooth. The individual variations of these successive measurements were ordinarily less than 6 per cent.

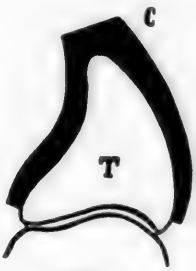

Fig. 2.

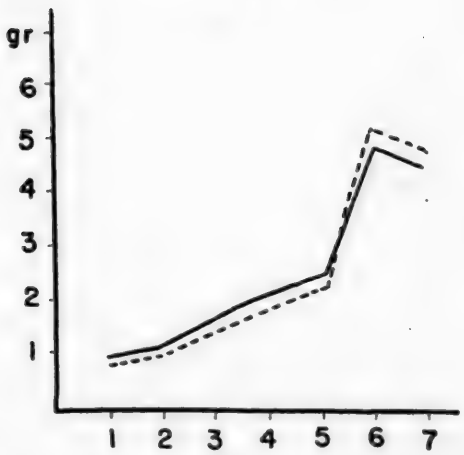

Fig. 3.—Normal thresholds to pressure.

Maxillary teeth: continuous line; mandibular teeth: dotted line. Abscissa: spatial arrangement of teeth in anteroposterior direction = (1) first incisor, (2) second incisor, (3) canine, (4) first premolar, (5) second premolar, (6) first molar, (7) second molar.

Ordinate: average thresholds.

In the experimental series in which an attempt is made to determine indirectly the position of the dental pressoreceptors, the studied tooth *(T)* is covered with an individually casted metallic cap *(c)* (Fig. 2). This cap is cemented

with zinc-oxyphosphate cement to the buccal and lingual surfaces of the tooth. Thus the pressure is applied to the incisal surface of the cap coaxially to the tooth.

RESULTS

Thresholds of 155 normal teeth were determined. The results obtained in the maxilla are given in Table I. The pressure thresholds vary between 0.948 Gm. (first incisor) and 4.533 Gm. (second molar). The corresponding standard deviations of the mean and variation coefficients are shown in Table I. The values increase significantly from the first incisors to the first molars following the slope of the curve shown in Fig. 3. There is a slight decrease of the thresholds from the first to the second molar.

TABLE I

THRESHOLDS OF NORMAL TEETH—MAXILLA

TOOTH	CASES	THRESHOLD (G.)	σ_m	V.C. (%)
First incisor	15	0.948	0.103	10.8
Second incisor	15	1.094	0.155	14.1
Canine	15	1.811	0.213	11.7
First premolar	10	2.003	0.145	8.4
Second premolar	10	2.479	0.100	4.0
First molar	10	4.875	0.539	11.0
Second molar	10	4.533	0.416	9.0

σ_m = Standard deviation of the mean.
V.C. = Variation coefficient.
Statistical calculus according to an earlier paper.[5]

The thresholds of the mandibular teeth follow a similar course (Table II and Fig. 3, dotted line). The values found for the mandibular incisors, canines, and premolars are somewhat lower if compared with the corresponding ones of the upper jaw. Nevertheless this finding lacks statistical significance.

TABLE II

THRESHOLDS OF NORMAL TEETH—MANDIBLE

TOOTH	CASES	THRESHOLD (GM.)	σ_m	V.C. (%)
First incisor	10	0.768	0.117	15.2
Second incisor	10	0.978	0.152	15.5
Canine	10	1.341	0.196	14.6
First premolar	10	1.771	0.251	14.1
Second premolar	10	2.028	0.372	17.0
First molar	10	5.030	0.457	8.9
Second molar	10	4.740	0.578	12.1

The ability of the subject to localize the stimulated tooth is practically 100 per cent in anterior teeth. In posterior teeth this ability shows a tendency to decrease.

In order to establish whether the nerve endings responsible for pressure reception have an intradental or periodontal location, and, furthermore, bearing in mind that threshold determinations could be used in clinical diagnosis, experiments were performed to measure pressure thresholds of pulpless teeth. Twenty-one teeth were studied. X-ray examinations were made to prove complete re-

moval of the pulp. In Table III the individual thresholds of pulpless teeth are compared with the corresponding normal values of the same subject. All cases, without exception, show significant higher values for pulpless as compared with normal teeth. The observed average threshold elevation of pulpless as compared with the homologous normal teeth is 57 per cent. A comparative study between 15 normal and 10 pulpless first incisors reveals that the observed difference is statistically significant (Table IV).

TABLE III

COMPARATIVE STUDY OF THRESHOLDS OF NORMAL AND DENERVATED TEETH

TOOTH	NORMAL (GM.)	DENERVATED (GM.)
Upper first incisor	0.483	0.700
	0.625	0.825
	0.416	0.900
	1.483	2.333
	1.600	2.500
	1.250	1.816
	0.816	1.700
	0.950	1.400
	0.783	1.150
Upper second incisor	0.916	1.233
	0.200	0.387
	0.890	2.183
Upper canine	2.462	2.510
Upper first premolar	2.933	3.216
Upper second premolar	1.233	2.400
Lower first premolar	0.866	1.133
Lower second premolar	3.583	4.170
	0.333	0.650
	4.633	6.041
Lower first molar	2.500	6.500
	3.966	5.400

TABLE IV

COMPARATIVE STUDY OF THRESHOLDS OF NORMAL AND DENERVATED FIRST INCISOR OF DIFFERENT SUBJECTS

NORMAL				DENERVATED				SIGNIFICANT DIFFERENCE
THRESHOLD (GM.)	CASES	σ_m	V.C. (%)	THRESHOLD (GM.)	CASES	σ_m	V.C. (%)	
0.948	15	0.103	10.8	1.567	10	0.246	15.6	2.38

Mean variation in 36 homologous teeth of 5 subjects = 6%.

In order to obtain additional information on the location of the receptors, a comparative study of thresholds of normal teeth and those presented by the same teeth covered with a metallic cap was performed. The latter show an average increase of 127 per cent (± 33 per cent), as compared with the former. This difference is amply significant.

On the other hand a similar study on the thresholds of denervated teeth and the same teeth covered with a metallic cap showed no difference at all.

DISCUSSION

Regarded from an ideal theoretical viewpoint, a study of the pressoreceptive sensibility should reflect the excitability of the single receptor. True receptor thresholds are only achieved in isolated receptor preparations recording the discharged action potentials. Our results have to be considered as the perception integrated from the simultaneous discharge of many pressoreceptors.

The applied force is transmitted by the tooth in the direction of its longitudinal axis to the receptors which we shall tentatively assume, until further discussion, to be localized in the periodontium.*

That normal posterior teeth present higher thresholds than normal anterior teeth does not necessarily mean that the receptors of the former are less excitable than the latter, but rather have to be explained as the consequence of the division of force on a greater surface. As the stimulated surface of the periodontium increases from anterior to posterior teeth, if equal force is applied to all teeth, the force per surface unit, i.e., the pressure received by each receptor, amounts to less in posterior than in anterior teeth.

No attempt was made in our experiments to apply horizontal forces, considering that any force not parallel to the longitudinal axis of the tooth transforms the latter into a lever with considerable individual variations of arm and fulcrum. Even if, in view of the explained reasons, no systematic threshold determinations were performed, we wish to point out that horizontal forces of less than 0.50 mg. are already perceived.

The observed thresholds show that the tooth possesses a highly sensitive sensorial function. Such sensitive receptors are well adapted to start, maintain, and regulate masticatory reflexes, and furthermore to signal harmful pressures to the levels of conscience which might develop during mastication. It is probable that they play a role in the self-regulation of the masticatory neural mechanism.

The finding that pulpless teeth present significant higher thresholds than normal ones provides the dental practitioner with an interesting weapon for diagnosis.

Concerning this aspect, our results disagree with the earlier works of Stewart[9] and Pfaffmann,[6, 7] who did not find differences in sensibility between normal and denervated teeth. Stewart's method, as he himself pointed out, was too gross to obtain a satisfactory quantitative result. Pfaffmann's[6, 7] work on the cat using von Frey's hairs is not comparable with ours.

From a theoretical point of view our results on thresholds of pulpless teeth suggest two possibilities of interpretation with regard to the localization of the dental pressoreceptors: (a) these are located in the interior of the tooth as well as in the periodontium and (b) the nerve fibers, or part of them, which convey impulses from the periodontal receptors to the nerve centers follow an intradental course.

*Whether these receptor are stimulated by compression or traction or both is beyond the scope of this work. Recently, one of us (W. R. L.) has shown that stretch is an adequate stimulus for cutaneous pressoreceptors.[10]

Nevertheless, both explanations do not invalidate the possibility of fibers of the periodontal nerve supply being destroyed along with the intradental nerve fibers in pulp removal. This consideration led to the experiments comparing the thresholds of normal teeth with those of normal teeth covered with metallic caps. If the receptors were located exclusively in the periodontium, the pressure impinging on them would be the same whether the force is applied directly to the tooth or through its cap. On the other hand if we accept the existence of intradental pressoreceptors, the pressure acting on these elements would be less in the covered than in the bare teeth. Hence, an increase in threshold has to be expected in the former as compared with the latter.

In the light of the foregoing arguments, the observed increase of the thresholds presented by the covered teeth has to be considered as an indirect proof of the existence of intradental pressoreceptors. These receptors are either more excitable than the periodontal ones or they are quantitatively sufficient to allow spatial summation.

On the basis of the presented experiments, it would be unwise to give an answer to the question of how these intradental receptors are stimulated by tiny pressures such as result from the application of threshold stimuli to the hard dental structures.

Which are the structures responsible for pressure reception of the tooth? The histologic literature mentions various types of nerve endings in the periodontium: free arborized endings,[2, 3] reticular plexi,[2, 8] buttonlike endings,[2-4] end-rings fixed on connective fibers of the periodontium,[8] spiderlike endings,[3] coiled endings,[3] and spiral spindles.[1, 3] In the dentin free and annular endings have been described.[8]

We are unable for the present to decide which of these endings are pressoreceptors. The spiral and spiderlike types seem to be specially suggestive due to their external resemblance to the muscle spindle ending and Meissner's corpuscle, respectively.

SUMMARY

A new esthesiometer for general use for excitability measurements of pressoreceptors is described.

A study of the pressoreceptive sensibility of human teeth is made with the following results: (1) The average threshold value to pressure of teeth is 2.523 Gm., a value which permits the classification of the teeth among the sensorial structures of high sensitivity. (2) The thresholds increase significantly from the anterior to the posterior teeth in the maxilla (0.948 Gm. first incisor to 4.875 Gm. first molar). A similar increase is observed in the mandible. (3) The thresholds of pulpless teeth are significantly higher (57 per cent) as compared with normal ones, the measurement of pressoreceptive sensibility thus providing the practitioner with a weapon for diagnosis. (4) Evidence is given for the existence of intradental as well as periodontal pressoreceptors.

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IN VITRO EXPERIMENTS ON THE BACTERICIDAL EFFECT OF ZINC OXIDE EUGENOL CEMENT ON BACTERIA-CONTAINING DENTIN

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THE surfaces of a cavity may be considered from three aspects: (a) the peripheral surface, (b) the walls, and (c) the centripetal pulpal surface. When the retention-resistance form[6] has been prepared, the final fate of the restoration in respect to a and b will rest equally upon the operator and the research chemist who is responsible for the physicochemical properties of the filling material, i.e., abrasiveness, compressive strength, adaptability, expansion, shrinkage, etc. The walls have to be prepared in such a way that enamel and dentin are totally free from soft and discolored dentin. This postulate has never been disputed. But with the third aspect, c, a dispute arises, especially with a deep carious cavity and a sound, noninfected pulp.

Here we find two schools of thought. The one demands complete removal of decayed soft and discolored tissue.[52, 40, 37] The other recommends leaving a layer of infected dentin and disinfecting it, rather than risking an exposure of the pulp.[49, 43, 11, 18] Feiler,[16] McBride,[32] and Coolidge,[13] for example, advocated very conservative treatment in deep caries of children and adolescents, Black[6] was rather noncommittal, Bonsack[7-10] and Kraus [24-28] being the most emphatic representatives of the "natural pulp capping" method. In Bonsack's various publications a wealth of information is to be found on this subject.

Other investigators recommended sterilization or disinfection of the bottom of the cavity. Aqueous silver nitrate and ammoniacal silver solution have been used by Winzenried,[53] Rosenstein,[42] McGehee,[33] Charbeneau,[11] and others. This condition has been studied by Zander,[54-57] Hardwick,[22] Muntz, Dorfman, and Stephan,[38] and others. From their valuable in vitro and in vivo studies on silver nitrate and on Howe's solution it can be summarized that silver nitrate penetrates carious dentin for a distance deep enough to sterilize an infected layer left over the pulp up to 1.3 mm. (Muntz) or 1.09 mm. (Zander). The pulp does not seem to be severely affected.

Others emphasized the impregnation of the carious layer with copper sulfate (Roemer as quoted by Miller,[34] Fiala[17]) or xylol (Coebergh[12]). An aliphatic ester of silicic acid called Tiranal has been recommended by German authors. Nufer[41] reported to have treated successfully deep decay with a calcium compound, Endoxyl. Day,[14] Mayer,[31] and others recommended thymol for disinfecting and sterilizing carious cavities.

Furthermore, many so-called germicidal cements are in use, to which silver powder, silver, copper, or mercury salts (F. Müller[36]), or some quinine derivatives (Eisfeld[15]) are added. The aim of this procedure is to hinder or

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to delay the occurrence of secondary decay, which may sometimes be unavoidable, for example, due to the mechanical or chemical shortcomings of filling materials (recently investigated by Fischer,[19] Armstrong and Simon,[1] and Nelsen, Wolcott, and Paffenbarger[39]). Skinner,[48] on the other hand, expressed the opinion that no germicidal cement is needed provided the cavity has been sterilized properly. Seltzer,[44-47] Zander,[55] and Besic[4] reported the presence and survival of bacteria in the hard dentin at the bottom of filled cavities.

MATERIALS AND METHODS

Most investigators advocated painting or impregnating the contaminated dentin left on top of the sound pulp with a disinfectant or sterilizing agent immediately prior to the insertion of the final filling. My experiments[50, 51] and those of Bartels[2, 3] have demonstrated the bactericidal properties of zinc oxide eugenol cement and its components. This compound is the most commonly used sedative cement and temporary dressing in daily practice. Reports by Gurley and Van Huysen,[20, 21] James and Diefenbach,[23] Manley,[29, 30] Mosteller,[35] and others confirm the practical experience, that this compound is not harmful to sound, noninfected pulp in man and in dogs.

In order to evaluate the bactericidal effect of zinc oxide eugenol the following experiment was undertaken: Human lower incisors were decalcified in trichloroacetic acid 10.0, distilled water 90.0, sodium chloride 10.0, and formalin (40 per cent) 5 parts. This solution is a mild agent and was kind to the teeth, which were soft and flexible after about 2 weeks. Each tooth was then transferred into a test tube filled with sterile tap water in order not only to wash out the acid, but to keep it sterile as well. The tubes were shaken several times a day and the teeth were transferred under sterile conditions into fresh tubes filled with sterile tap water, until the water proved to be neutral (pH 7.0). Each tooth was then transferred into a new test tube filled with sterile glucose broth and tested for sterility by being kept in the incubator for 48 hours. In order to make absolutely sure that there was no acid left in the centers of the teeth, they were placed into a second sterile test tube containing glucose broth. The first test tube containing broth only was inoculated with a test strain, and when growth occurred after 24 hours this was taken as a sign that the decalcified tooth, so far sterile, was then in a suitable condition to be experimentally "infected." With the use of sterile instruments the tooth was removed from the broth and with a sterile razor blade it was dissected horizontally into slices approximately 1 mm. thick. The crown and the apices of the tooth were left out. The other discs were placed each one into one Kahn tube, containing 1 ml. of sterile glucose broth. They were tested for sterility for 48 hours. A few, apparently contaminated, were then discarded. Then each sterile tube, containing one slice of decalcified root in broth, was heavily inoculated with one loop of a 48-hour-old glucose agar culture of the following test strains: (1) *Staphylococcus pyogenes aureus* (Strain 6832 of the National Collection of Type Cultures), (2) *Lactobacillus acidophilus odontolyticus* (Strain 1406), (3) *Escherichia coli* (Strain 86), and (4) *Monilia*

albicans (Strain Z 248). In this manner 12 tubes of each strain (48 tubes in all) were inoculated and incubated for 72 hours. After this time it could be presumed that every root disc was thoroughly impregnated with 1 test strain.

Meanwhile discarded cartridges used in local anesthesia were prepared as follows: After they were cleaned and all traces of chemicals were washed out, the piercing diaphragm was removed and replaced by an absorbent cotton plug. In the other end of the cartridge the rubber stopper was inserted, approximately 5 mm. inside the glass tube, leaving a free space which was plugged with absorbent cotton. The cartridge was then wrapped in cellophane and sterilized by autoclaving at 15 pounds for 15 minutes.

With the use of a sterile needle, an inoculated root slice was removed from the Kahn tube and placed on the rubber plunger after removal of the cotton plug, and the "cavity" over the contaminated dentin was immediately filled with a zinc oxide eugenol cement. The analysis of the zinc oxide used in this experiment is as follows:

Carbonate (CO_3)	none	
Chloride (Cl)	0.002	per cent
Sulfate (SO_4)	0.008	per cent
Iron (Fe)	0.0001	per cent
Lead (Pb)	0.002	per cent
Metallic zinc (Zn)	none	
Oxidizable matter	0.0012	per cent
Arsenic (As_2O_3)	absent.	

TABLE I

STRAIN	ONE-HOUR TEST	THREE-HOUR TEST	THREE-HOUR PLASTER OF PARIS CONTROL	SIX-HOUR TEST	TWENTY-FOUR-HOUR TEST	THIRTY-TWO-HOUR TEST	FORTY-EIGHT-HOUR TEST	FORTY-EIGHT-HOUR PLASTER OF PARIS CONTROL	AGAR CONTROL
6832	– – –	+	+ + +	– – –	– – –	– – –	– – –	+ + +	+ + +
1406	+ +	– – –	+ + +	– – –	– – –	– – –	– – –	+ + +	+ + +
86	– – –	– – –	+ + +	– – –	– – –	– – –	– – –	+ + +	+ + +
248	+ + +	+	+ + +	+ +	– – –	Test	– – –	+ + +	+ + +
					– – –	omitted			
					(2 tests)				

– – – Indicates no growth.
+ } Indicates scanty growth.
+ + }
+ + + Indicates normal growth.

Five-tenths gram of zinc oxide was thoroughly mixed with 0.1 ml. of eugenol (British Pharmaceutical Codex—1949) until a stiff paste resulted. This paste resembled a temporary dressing. It was tightly packed over the inoculated dentin.

The purpose of these experiments was to evaluate if and in what time contaminated dentin can be sterilized by zinc oxide eugenol cement. The cement was left in close contact (in a moist chamber and in the incubator) for 1, 3, 6, 24, 32, and 48 hours, respectively. Controls consisted of inoculated

slices being placed on the rubber plunger inside the cartridge, but instead of zinc oxide eugenol cement, a sterile plug of plaster of Paris and water was employed for 3 and 48 hours, respectively.

After the appropriate time had elapsed, each cartridge was removed from the incubator, placed in a sterile bag of kraft paper, and crushed with a hammer. The bags were opened and the slices of inoculated dentin were recovered with sterile needles, and transferred into sterile broths. After an incubation period of from 48 hours to 21 days, the tubes were tested for sterility or growth by subculturing and microscopic examination. The results can be seen from Table I.

In another series it was attempted to evaluate the bactericidal or bacteriostatic effect of zinc oxide eugenol cement on natural carious tissue, in vitro. Preferably, extracted molars with extensive decay were chosen, to provide large "chunks" of soft tissue. The technical procedure was the same as described previously. The carious material was left under the cement for 13, 15, 25, 36, 48, 59, and 74 hours, respectively. None of these experiments was negative; i.e., in no single case did the cement show any bactericidal effect.

DISCUSSION

After the test strain series was completed the decalcified root discs were measured for thickness. They averaged 0.1 cm. Their average length was 0.51 cm. and their width, 0.3 cm. No data can be given about their special histologic or physical structure regarding resistance to infection.

Strain 6832 (*Staph. pyogen. aur.*) was obviously affected by cement, as no growth occurred after contact of 1 hour; after 3 hours' influence of the cement, the inoculated dentin disc produced mild opalescence in the broth, ie., the cement proved to be bacteriostatic. After 6 hours no growth could be observed. One test tube containing the inoculated dentin, after 32 hours' contact following an incubation of 3 weeks, turned slightly cloudy on shaking when compared with clear sterile broth. Microscopic examination and subculture on glucose agar were negative. The stained slide showed unorganized matter.

Strain 1406 (*Lactobacillus acidophil. odontolyt.*) was not killed after 1 hour's contact with the cement, but after 3 hours' influence the dentin was sterile and remained so after 6, 24, 32, and 48 hours, respectively.

The gram-negative strain 86 (*Escherichia coli*) was the most sensitive organism in this series, as it was killed after only 1 hour's contact with the zinc oxide eugenol cement.

Strain Z 248 (*Candida albicans*), which is resistant to most antibiotics, was certainly affected by the cement; although full growth occurred immediately after 1-hour contact, the 3- and 6-hour tests proved to be negative in the beginning; i.e., the slices transferred into broth did not appear to have resulted in growth. After 21 days, growth occurred. This growth proves that a few cells must have survived and multiplied, perhaps those in the center of the contaminated dentin disc. This would be a condition which some writers de-

scribe as "almost sterile." On the other hand, the 2 tests of 24 and 48 hours' contact proved negative. Here the broth remained sterile, the resistant yeast cells having been killed.

The controls under a plaster of Paris plug were all positive. The inoculated slices recovered after 3 hours' contact with plaster of Paris were soft, as were all the other slices used in these experiments with the exception of the 1 control, where the plaster of Paris rested for 48 hours over the dentin disc. Here the slices were hard and shriveled and it was expected that the bacteria might have dried out and died. Bigger's[5] words may be remembered here. He stated that it is very often more for the philosopher than for the bacteriologist to decide whether bacteria have died by a substance or in its presence, as they have the tendency to die if they are prevented from growing.

The evaluation of the "natural decay" series was extended to a microscopic analysis. The broth into which the carious tissue after the contact with the zinc oxide eugenol cement was transferred was observed for several days. The findings for tube No. 1, for example, read as follows: Decay transferred after 15 hours' contact with cement. The next day, after 25 hours in broth, the tube was clear, but became cloudy after shaking; microscopically visible were a few yeasts and lactobacilli, recognizable by formation of palisades, one chain of cocci, clusters of plump diplococci, and a chain of approximately 15 plump rods. After a further 24 hours' incubation there appeared chains of plump bacilli, a few staphylococci, and a few lactobacilli, but no yeast. After another 24 hours: chains of plump bacilli, chains of slender bacilli, some streptobacilli, no yeast. The findings in the 6 other tubes are somewhat similar. Interesting is the disappearance of yeasts, an organism which usually overgrows other bacteria in vitro. It has been shown that yeasts are sensitive to some essential oils (Bartels[2]).

The discrepancy in the different reaction of the test strains and the natural decay to the zinc oxide eugenol cement may be explained, for example, by the attenuation of the strains through countless subcultures and through growth on an artificial medium.

SUMMARY

1. Sterile slices of decalcified dentin, 1 mm. thick, were inoculated with 4 pure strains of *Staphyl. pyogenes aureus, Latobacillus acidophilus odontolyt., E. coli, and Candida albicans* and exposed to a zinc oxide eugenol cement in vitro, for 1, 3, 6, 24, 32, and 48 hours, respectively.

2. Gram-negative *E. coli* proved to be the most sensitive organism throughout the entire series.

3. The cement exerted a bacteriostatic and bactericidal effect on the 3 other strains.

4. After close contact with the cement for 24 and 48 hours, all inoculated slices were sterile.

5. Under similar conditions, natural decay obtained from extracted carious teeth was resistant and in no single case was the decayed dentin sterilized.

6. It is, therefore, intended to reinforce this cement in such a way that it will disinfect, if not sterilize, carious tissue in vitro as well as in vivo without impairing the vitality of the pulp.

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